Notices

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In This Guide...

This manual covers the Agilent 1290 Infinity II Method Development Solution, which consists of the Agilent 1200 Infinity I/II Series Method Development System and the Agilent ChemStation Method Scouting Wizard.

1 Method Development Solution

This chapter gives information on Prerequisites for the Method Development Solution.

2 The Agilent 1290 Infinity II Method Development System

This chapter gives information on system components, column and solvent switching.

3 System Setup and Installation

This chapter provides information on system setup, installation of valve heads, heat exchangers and capillaries, and installation of solvent selection parts.

4 Configuring the System in ChemStation and Creating Methods

This chapter explains how to configure the system in the control software and how to create methods.

5 The Agilent ChemStation Method Scouting Wizard

This chapter provides information on installation, use and features of the software.

6 Method Development Strategy

This chapter provides information on method development strategy, concerning LC and LC/MS columns selection, pH and mobile phase.

7 Appendix

This chapter provides addition information on safety, legal and web.
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This chapter gives information on Prerequisites for the Method Development Solution.
High Pressure Liquid Chromatography (HPLC) allows the efficient separation of compounds and therefore is a key technology in pharmacology and chemistry. The interaction of analyte molecules with so-called stationary and mobile phases determines the compound separation. Developing the optimal method for different compounds can be quite demanding. There are almost endless possible combinations of crucial parameters like solvent type, column type, temperature, and gradient.

The Agilent Method Development Solution (MDS) allows simple generation of a sequence that varies all crucial method parameters systematically. The MDS is based on a skilled soft- and hardware configuration.

**Hardware Configuration**

The Agilent 1200 Infinity Series Multi-method and Method Development System consists of an Agilent 1200 Infinity II Series LC or an Agilent 1200 Infinity Series LC with the following options:

- Single Multicolumn Thermostat (MCT) solution with one MCT module.
- A valve thermostat cluster (VTC) with multiple valve hosts (Multicolumn Thermostats, Valve Drives, Thermostatted Column Compartments) and temperature zones (Multicolumn Thermostats, Thermostatted Column Compartments, Integrated Column Compartments).

(For a list of all supported modules see Table 1 on page 12.)

Functions of the hardware:

- Solvent delivery that is combined with external solvent selection:
  - Variation of liquid phase and generation of gradients
- Column compartment:
  - Determination of temperature
- Column selection
  - Variation of stationary phase
Software Configuration

The ChemStation Method Scouting Wizard A.02.07 is an Add-on for OpenLAB CDS ChemStation.

The Method Scouting Wizard automatically generates all steps to flush the system with any required solvents, performs column equilibration procedures, and defines storage conditions for columns in predefined storage solvents. In this respect, it uses waste and/or available bypass lines intelligently to allow fast flushing procedures. Flushing, equilibration and column storage procedures, and temperature changes are arranged in the workflow such that a minimal number of these steps need to be performed to save valuable time and solvents. The Method Scouting Wizard requires the following software components:

- OpenLAB CDS ChemStation Edition C.01.07 SR3, Workstation

**NOTE**

Not supported: Secure Workstation and Distributed AIC

- LC & CE Drivers A.02.16

**NOTE**

Make sure that driver version A.02.16 is installed:

- Check the installed driver version with your OpenLAB CDS ChemStation Edition C.01.07 SR3.
- Upgrade or downgrade if the driver version is different.

For details on the possible hardware configurations, see Table 1 on page 12.

For details on the software requirements, see “Overview of the Agilent Method Scouting Wizard” on page 68.
Method Development Solution

Method Development Solution
2 The Agilent 1290 Infinity II Method Development System

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This chapter gives information on system components, column and solvent switching.
System Components

Description of the System

The hardware of the Agilent 1200 Infinity Series Multi-method and Method Development System consists of an Agilent 1200 Infinity II Series LC or an Agilent 1200 Infinity Series LC. The system components are listed in Table 1 on page 12.

Table 1 MDS System Components

<table>
<thead>
<tr>
<th>Function</th>
<th>Module</th>
<th>Description</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent delivery</td>
<td>G1311B</td>
<td>1260 Infinity Quaternary Pump, 600 bar</td>
<td>Equipped with built-in degasser</td>
</tr>
<tr>
<td></td>
<td>G1312B</td>
<td>1260 Infinity Binary Pump, 600 bar</td>
<td>Requires external degasser</td>
</tr>
<tr>
<td></td>
<td>G4204A</td>
<td>1290 Infinity Quaternary Pump, 1200 bar</td>
<td>Equipped with built-in degasser</td>
</tr>
<tr>
<td></td>
<td>G4220A</td>
<td>1290 Infinity Binary Pump, 1200 bar</td>
<td>Equipped with built-in degasser</td>
</tr>
<tr>
<td></td>
<td>G4220B</td>
<td>1290 Infinity Binary Pump VL</td>
<td>Equipped with built-in degasser</td>
</tr>
<tr>
<td></td>
<td>G7104A</td>
<td>1290 Infinity II Flexible Pump 1300 bar</td>
<td>Equipped with built-in degasser</td>
</tr>
<tr>
<td></td>
<td>G7120A</td>
<td>1290 Infinity II High Speed Pump 1300 bar</td>
<td>Equipped with built-in degasser</td>
</tr>
<tr>
<td></td>
<td>G7111A</td>
<td>1260 Infinity II Quaternary Pump VL, 400 bar</td>
<td>Equipped with built-in degasser</td>
</tr>
<tr>
<td></td>
<td>G7111B</td>
<td>1260 Infinity II Quaternary Pump, 600 bar</td>
<td>Equipped with built-in degasser</td>
</tr>
<tr>
<td></td>
<td>G4302A</td>
<td>1260 Infinity SFC Binary Pump</td>
<td>Requires external degasser</td>
</tr>
<tr>
<td></td>
<td>G5611A</td>
<td>1260 Infinity Bio-inert Quaternary Pump, 600 bar</td>
<td>Equipped with built-in degasser</td>
</tr>
</tbody>
</table>
## System Components

### External solvent selection
- **G1170A 1290 Infinity Valve Drive**
- **G4235A 12-Pos/13-Port Solvent Selection Valve Head**
  - Additional tubing kits required (Solvent selection tubing kit, 4 solvents (5067-4601))

### Sample introduction
Any of the Agilent 1200 Infinity Series Autosamplers supported by LC&CE Drivers A.02.16 can be used. Select your autosampler suitable to the required maximum pressure capability of the system.

The following new modules/options are available:

- **G7129A 1260 Infinity II Vialsampler**
- **G7129B 1290 Infinity II Vialsampler**
- **G7167A 1260 Infinity II Multisampler**
  - Including dual needle option
- **G7167B 1290 Infinity II Multisampler**
  - Including dual needle option
- **G7116B 1290 Infinity II Multicolumn Thermostat**
- **G7116A 1260 Infinity II Multicolumn Thermostat**
- **G7130A 1290 Infinity II Integrated Column Compartment**
  - Used with G7129A/B
- **G1316C 1290 Infinity Thermostatted Column Compartment**
- **G1316A 1260 Infinity Thermostatted Column Compartment**
  - No low dispersion heat exchangers possible
  - Only as third Thermostatted Column Compartment for additional space

### Table 1  MDS System Components

<table>
<thead>
<tr>
<th>Function</th>
<th>Module</th>
<th>Description</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>External solvent selection</td>
<td>G1170A</td>
<td>1290 Infinity Valve Drive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G4235A</td>
<td>12-Pos/13-Port Solvent Selection Valve Head</td>
<td>Additional tubing kits required (Solvent selection tubing kit, 4 solvents (5067-4601))</td>
</tr>
<tr>
<td>Sample introduction</td>
<td>G7129A</td>
<td>1260 Infinity II Vialsampler</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G7129B</td>
<td>1290 Infinity II Vialsampler</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G7167A</td>
<td>1260 Infinity II Multisampler</td>
<td>Including dual needle option</td>
</tr>
<tr>
<td></td>
<td>G7167B</td>
<td>1290 Infinity II Multisampler</td>
<td>Including dual needle option</td>
</tr>
<tr>
<td>Column compartment</td>
<td>G7116B</td>
<td>1290 Infinity II Multicolumn Thermostat</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G7116A</td>
<td>1260 Infinity II Multicolumn Thermostat</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G7130A</td>
<td>1290 Infinity II Integrated Column Compartment</td>
<td>Used with G7129A/B</td>
</tr>
<tr>
<td></td>
<td>G1316C</td>
<td>1290 Infinity Thermostatted Column Compartment</td>
<td></td>
</tr>
</tbody>
</table>
|                        | G1316A  | 1260 Infinity Thermostatted Column Compartment | No low dispersion heat exchangers possible
                                                                         | Only as third Thermostatted Column Compartment for additional space |
The Agilent 1290 Infinity II Method Development System

System Components

Table 1  MDS System Components

<table>
<thead>
<tr>
<th>Function</th>
<th>Module</th>
<th>Description</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column selection</td>
<td>G4239C</td>
<td>8-column selection valve head, 1300 bar (for installation in G7116B)</td>
<td>One 8-Pos/18-Port Valve Head 1300 bar</td>
</tr>
<tr>
<td></td>
<td>G4234A</td>
<td>Multi-column selection valve head, 600 bar (for installation in G1316C or G1170A)</td>
<td>One 6-Pos/14-Port Valve Head 600 bar</td>
</tr>
<tr>
<td></td>
<td>G4234B</td>
<td>Multi-column selection valve head, 1200 bar (for installation in G1316C or G1170A)</td>
<td>One 6-Pos/14-Port Valve Head 1200 bar</td>
</tr>
<tr>
<td></td>
<td>G4237A</td>
<td>4-Column selector valve kit, 600 bar (for installation in G7116A)</td>
<td>One 4-Pos/10-Port Valve Head 600 bar</td>
</tr>
<tr>
<td></td>
<td>G5639A</td>
<td>4-Column selector valve kit Bio-inert, 600 bar (for installation in G7116A)</td>
<td>One 4-Pos/10-Port Valve Head Bio-inert 600 bar</td>
</tr>
<tr>
<td></td>
<td>G4230A</td>
<td>Method Dev. Valve Kit, 600 bar (for installation in G1316C)</td>
<td>Two 8-Pos/9-Port Valve Heads 600 bar</td>
</tr>
<tr>
<td></td>
<td>G4230B</td>
<td>Method Dev. Valve Kit, 1200 bar (for installation in G1316C)</td>
<td>One 8-Pos/9-Port Valve Head 600 bar</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>One 8-Pos/9-Port Valve Head 1200 bar</td>
</tr>
</tbody>
</table>
Solvent delivery

Binary pumps are equipped with an internal solvent selection valve allowing two different solvents per pump channel (default for G7120A and G4220A, optional for G1312B).

For increased solvent selection capability each pump can additionally be equipped with two external solvent selection valves that extend the number of available solvents on one pump channel to a maximum of 12, see Figure 1 on page 15.

Figure 1    Method development system principle with external solvent solution
System Components

**External solvent selection**

The external solvent selection valve consists of a 1290 Infinity Valve Drive (G1170A) in combination with a G4235A 12-Pos/13-Port Solvent Selection Valve Head.

If two external solvent selection valves are configured, they must both be connected either before or after the degasser. Connection of one valve on either side of the degasser is not supported.

For further details and examples refer to chapter *System Setup and Installation*.

**Sample introduction**

Any of the Agilent 1200 Infinity Series Autosamplers supported by LC&CE Drivers A.02.16 can be used (see Table 1 on page 12). Select your autosampler suitable to the required maximum pressure capability of the system.

**Column compartments**

For the temperature zones there is a great variety of possibilities. Up to eight short (or four long or four short and two long...) columns can be used with one G7116B Multicolumn Thermostat (single MCT). With a valve thermostat cluster (VTC) of up to four G7116B Multicolumn Thermostat modules it is possible to use up to 32 columns. In addition to that, it is possible to combine an MCT with other components such as ICC (G7130A).

For further details and examples refer to chapter *System Setup and Installation*.

**Column selection**

Several valve hosts like G7116A (MCT), G7116B (MCT) or G1170A (1290 Infinity Valve Drive) can be used for column selection. For the compatible valves heads/valve kits see Table 1 on page 12.
Sample detection and analysis

A very broad range of detectors is supported, including light detectors (UV, DAD, FLD), mass selective detectors, evaporative light scattering detectors and refractive index detectors. Other detectors, for example chiral detectors, can be used by acquiring the signal via an analog to digital converter.

Capillary Kits

For use with MCT the following capillary kit is available:

- Capillary Kit, 0.12 mm (5067-4248) for 8-column selector with Quick-Connect heat exchangers (standard flow), see “Infinity II Capillary Kit” on page 24

For use with TCC the following capillary kits are available:

- Cap Kit 0.12 mm LDHE double 8/9 vlv short col (5067-6220), see “Low Dispersion Capillary Kit for Short Columns” on page 30
- Cap Kit 0.12 mm LDHE double 8/9 vlv long col (5067-4289), see “Low Dispersion Capillary Kit for Long Columns” on page 32
- Cap Kit 0.17 mm 8/9 multi purpose (5067-4290), see “General Purpose Capillary Kit” on page 31

The capillary kit that is used depends on the columns to be installed.

All kits, and all components of the kits, can be ordered separately as consumables.
Solvent Switching

In addition to the two or four solvents directly supported by the pump, up to two ports of the pump can be connected to an external Valve Drive (G1170A) equipped with a 12-position/13-port solvent selection valve head (G4235A). This valve is able to switch between up to 12 solvents giving a total of up to 26 solvents on the Agilent 1200 Infinity Series LC Multi-method and Method Development System.

An overview of the components for solvent selection is given in “Components for Solvent Selection (Agilent Infinity-II LC)” on page 19, “Components for Solvent Selection (Agilent 1290 Infinity LC)” on page 20, and “Components for Solvent Selection (Agilent 1260 Infinity LC)” on page 22.

All Agilent pumps are delivered together with one solvent cabinet and as many solvent reservoirs as available solvent channels of the pump (two or four). For Agilent 1260 Infinity Binary Pumps the built-in solvent selection option is recommended.

For the selection of the degasser and the number of degassers in a system, refer to “Deciding on the Position of the Degasser” on page 45.
Components for Solvent Selection (Agilent Infinity-II LC)

<table>
<thead>
<tr>
<th>Item</th>
<th>p/n</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5067-4601</td>
<td>Solvent selection tubing kit, 4 solvents</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kit 5067-4601 contains short tubings for setup as shown.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Single stack setup (1290 Infinity and Infinity II) with long tubing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>connections to pump requires Infinity II bottle head assemblies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G7120-60007 plus 0100-2298 Adapter, PEEK int. 1/4-28 to ext. 10-32 per channel.</td>
</tr>
<tr>
<td>2</td>
<td>G1170A</td>
<td>1290 Infinity Valve Drive in combination with 12pos/13port valve, bio-inert (G4235A)</td>
</tr>
<tr>
<td>3</td>
<td>5067-5760</td>
<td>Solvent Cabinet Kit</td>
</tr>
<tr>
<td>4</td>
<td>9301-1450</td>
<td>Solvent bottle, amber</td>
</tr>
<tr>
<td>OR</td>
<td>4</td>
<td>9301-1420 Solvent bottle, transparent</td>
</tr>
</tbody>
</table>

**NOTE**

One solvent cabinet and bottles are included with the pump.
Components for Solvent Selection (Agilent 1290 Infinity LC)

Figure 3 Components for solvent selection (Agilent 1290 Infinity LC)
### Solvent Switching

<table>
<thead>
<tr>
<th>Item</th>
<th>p/n</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5067-4601</td>
<td>Solvent selection tubing kit, 4 solvents&lt;br&gt;Up to 4 required. Kit 5067-4601 contains short tubings for setup as shown. Single stack setup (1290 Infinity and Infinity II) with long tubing connections to pump requires Infinity II bottle head assemblies G7120-60007 plus 0100-2298 Adapter, PEEK int. 1/4-28 to ext. 10-32 per channel.</td>
</tr>
<tr>
<td>2</td>
<td>G1170A</td>
<td>1290 Infinity Valve Drive in combination with 12pos/13port valve, bio-inert (G4235A)</td>
</tr>
<tr>
<td></td>
<td>5067-4634</td>
<td>Valve rail assembly</td>
</tr>
<tr>
<td>3</td>
<td>5065-9981</td>
<td>Solvent cabinet 1200 Infinity, including all plastic parts</td>
</tr>
<tr>
<td>4</td>
<td>9301-1420</td>
<td>Solvent bottle, transparent</td>
</tr>
<tr>
<td>OR 4</td>
<td>9301-1450</td>
<td>Solvent bottle, amber</td>
</tr>
</tbody>
</table>

**NOTE**<br>One solvent cabinet and bottles are included with the pump.
The G1311B Quaternary Pump has a built-in degasser. An additional external degasser is required for G1312B Binary Pumps.
The Agilent 1290 Infinity II Method Development System

Solvent Switching

Components of the Tubing Kit

The tubing kit (Solvent selection tubing kit, 4 solvents (5067-4601)) contains the following items:

<table>
<thead>
<tr>
<th>Item</th>
<th>#</th>
<th>p/n</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>5062-2483</td>
<td>Tube PTFE 1.5 mm x 5 m, 3 mm od</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0100-2298</td>
<td>Adapter, PEEK int. 1/4-28 to ext. 10-32</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>G1311-60003</td>
<td>Bottle-head assembly</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>5063-6598</td>
<td>Tefzel ferrules and SSL lock rings, 1/8 inch, 10/pck</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>5063-6599</td>
<td>PPS nuts, 1/8 inch, 1/4-28 thread, 10/pck</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>5042-9954</td>
<td>Tubing clip (2x), re-order 4/pk</td>
</tr>
</tbody>
</table>

One solvent cabinet and bottles are included with the pump.
Column Selection

Column Selection with one G7116B (single MCT)

To perform automated column switching with the Agilent 1200 Infinity Series Multi-method and Method Development System, one 1290 Infinity II Multicolumn Thermostat equipped with a G4239C 8-column selector valve head is required. A maximum of eight positions are available to connect columns, bypass, or waste lines. By using the Agilent low dispersion heat exchanger, up to eight solvent streams can be pre-heated to a maximum of 110 °C, depending on the flow rate.

Infinity II Capillary Kit

<table>
<thead>
<tr>
<th>p/n</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5067-4233</td>
<td>8 Column Selector, 1300 bar</td>
</tr>
<tr>
<td>5067-4248</td>
<td>Capillary Kit, 0.12 mm for 8-column selector with Quick-Connect heat exchangers (standard flow)</td>
</tr>
<tr>
<td>5500-1202</td>
<td>Capillary SST 0.12x500mm M4-SL PS-PS</td>
</tr>
<tr>
<td>5500-1199</td>
<td>Capillary SST 0.12x130mm M4-SL PS-PS</td>
</tr>
<tr>
<td>5500-1200</td>
<td>Capillary SST 0.12x130mm M4 PS-NS LS</td>
</tr>
<tr>
<td>5063-6591</td>
<td>PEEK Fittings 10/PK</td>
</tr>
<tr>
<td>5500-1201</td>
<td>Capillary SST 0.12x105mm SL-- PS-LS</td>
</tr>
<tr>
<td>G1314-68703</td>
<td>Cap fitting kit special</td>
</tr>
<tr>
<td>5500-1203</td>
<td>Capillary SST 0.12x280mm M4-SL PS-PS</td>
</tr>
<tr>
<td>5500-1204</td>
<td>Capillary SST 0.12x150mm M4-M4 PS-PS</td>
</tr>
<tr>
<td>5023-2504</td>
<td>Hex driver SW-4 slitted</td>
</tr>
<tr>
<td>G7116-60015</td>
<td>Heat Exchanger Assembly 1.6 µL-Z Quick Connect Heatexchanger Standard Flow</td>
</tr>
<tr>
<td>G1375-87326</td>
<td>Waste tube</td>
</tr>
<tr>
<td>5067-6141</td>
<td>M4 Blank nut</td>
</tr>
<tr>
<td>G7116-68003</td>
<td>Column Holder Clips (2/Pk) for G7116B</td>
</tr>
</tbody>
</table>
Column Selection with one G7116A (single MCT)

To perform automated column switching with the Agilent 1200 Infinity Series Multi-method and Method Development System, one 1260 Infinity II Multicolumn Thermostat equipped with a 4-column selector valve head is required. A maximum of four positions are available to connect columns, bypass, or waste lines.

<table>
<thead>
<tr>
<th>p/n</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>G4237A</td>
<td>4 column selector valve kit</td>
</tr>
<tr>
<td>G5639A</td>
<td>4 column selector valve kit, bio-inert</td>
</tr>
</tbody>
</table>
Column Selection in a Valve Thermostat Cluster (VTC)

Column Selection in a Valve Thermostat Cluster (VTC)

A valve thermostat cluster (VTC) is a powerful solution for using up to 32 columns in a system. Different topologies allow multiple combinations of valve hosts (Multicolumn Thermostats, Valve Drives, Thermostatted Column Compartments) and temperature zones (Multicolumn Thermostats, Thermostatted Column Compartments, Integrated Column Compartments). The recommended VTC topologies are described in the following.

**NOTE**

Agilent 1260 Infinity II Multicolumn Thermostat (G7116A) does not support clustering!

A Valve Thermostat Cluster (VTC) for long columns

This most simple VTC topology is needed for clustering one valve with a column host (one or two Column Compartments). With two MCT it is possible to use up to 8 long columns (or any combinations of long and short columns).

It is also possible to use this topology with a valve in an external valve drive and an ICC.
A Valve Thermostat Cluster (VTC) for up to 32 Columns

In the valve thermostat cluster, the described VTC solution (see “A Valve Thermostat Cluster (VTC) for long columns” on page 26) can be multiplicated up to four times. These branched topologies are typically realized via an external valve and some MCT modules, each equipped with an 8-column selection valve.

NOTE
The number of branches is fix. If a topology with four branches is selected, four valves have to be configured in the branches - plus one branching valve (root valve).
Column Selection with two to three TCC

To perform automated column switching with the Agilent 1200 Infinity Series Multi-method and Method Development System, two 1290 Infinity Thermostatted Column Compartments (G1316C), each equipped with an 8pos/9port valve, are required. A maximum of eight positions are available to connect columns, bypass, or waste lines. By using the Agilent low dispersion heat exchanger, up to eight solvent streams can be pre-heated to a maximum of 100 °C (G1316C), depending on the flow rate. If six columns longer than 100 mm have to be installed and solvent pre-heating is required, either 3x G1316C or 2x G1316C and 1x G1316A/B are necessary.

For automated column switching, the following parts and modules have to be integrated:

Figure 5  Modules and parts needed for column switching
Two different valve kits are available:

- The G4230A valve kit is required for standard Agilent LCs and contains two 8-position/9-port valve heads (Valve Head 8 Position/9 Port, 600 bar (5067-4107)) rated to a maximum pressure of 600 bar.

- The G4230B valve kit is required for an Agilent 1290 Infinity LC, and contains two different valve heads:
  - On the column inlet side, an ultra-high-pressure-rated 8-position/9-port valve head that is rated to a maximum pressure of 1200 bar (Valve Head 8 Position/9 Port, 1200 bar (5067-4121)) is necessary;
  - on the column outlet side, only little backpressure is given and a standard 600 bar selection valve (Valve Head 8 Position/9 Port, 600 bar (5067-4107)) is sufficient.

One of three available capillary kits is delivered along with the valve kit.
### Infinity Capillary Kits

#### Low Dispersion Capillary Kit for Short Columns

The components of the low dispersion capillary kit for short columns (Cap Kit 0.12 mm LDHE double 8/9 vlv short col (5067-6220)) are listed below.

Valve 1 refers to the inlet valve and Valve 2 refers to the outlet valve.

<table>
<thead>
<tr>
<th>#</th>
<th>p/n</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>G1316-60005</td>
<td>LD-Pre-Column Heatexchanger Double-Assy</td>
</tr>
<tr>
<td>8</td>
<td>5500-1201</td>
<td>Capillary SST 0.12x105mm SL-- PS-LS Heatexchanger to column, SL fitting pre-swaged</td>
</tr>
<tr>
<td>1</td>
<td>G1316-90123</td>
<td>Technical Note <em>(Installation of the Low Dispersion Heat-Exchanger Double Assemblies in the 1290 Series Thermostatted Column Compartment (G1316C), ENG)</em></td>
</tr>
<tr>
<td>8</td>
<td>G7167-68703</td>
<td>Fitting Intermediate Kit</td>
</tr>
<tr>
<td>1</td>
<td>G1316-87319</td>
<td>Capillary ST 0.12 mm x 340 mm S/S Autosampler with thermostat to valve 1</td>
</tr>
<tr>
<td>1</td>
<td>5500-1157</td>
<td>Capillary, ST, 0.12 mmx500 mm Autosampler to valve 1 in a dual stack configuration</td>
</tr>
<tr>
<td>8</td>
<td>5067-4604</td>
<td>Capillary ST 0.12 mm x 280 mm S/SX Valve 1 to heat-exchanger</td>
</tr>
<tr>
<td>8</td>
<td>5067-1191</td>
<td>Capillary ST 0.12 x 280 mm, long socket Column outlet to valve 2, capillary w.o. fittings, use PEEK fingertight</td>
</tr>
<tr>
<td>2</td>
<td>0890-1713</td>
<td>Waste tubing, 2 m</td>
</tr>
<tr>
<td>1</td>
<td>5042-9918</td>
<td>Column clip set, eight colors</td>
</tr>
<tr>
<td>1</td>
<td>5041-2115</td>
<td>Folding box</td>
</tr>
<tr>
<td>1</td>
<td>5063-6591</td>
<td>PEEK Fittings 10/PK</td>
</tr>
<tr>
<td>1</td>
<td>5062-8541</td>
<td>Fingertight fitting long 10/pk Column outlet capillary valve 2 ports 4</td>
</tr>
</tbody>
</table>
General Purpose Capillary Kit

The components of the general purpose capillary kit (Cap Kit 0.17 mm 8/9 multi purpose (5067-4290)) are listed below.

Valve 1 refers to the inlet valve and Valve 2 refers to the outlet valve.

The general purpose capillary kit is not recommended for 1290 Infinity LCs.

<table>
<thead>
<tr>
<th>#</th>
<th>p/n</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>5500-1196</td>
<td>Capillary ST 0.17 mm x 280 mm, long socket</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Autosampler to TCC heater, vlv-det</td>
</tr>
<tr>
<td>1</td>
<td>5500-1236</td>
<td>Capillary ST 0.17 mm x 400 mm, long socket</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ALS therm-TCC heater</td>
</tr>
<tr>
<td>1</td>
<td>5065-9933</td>
<td>Capillary ST 0.17 mm x 600 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Autosampler to TCC heater in a dual stack configuration</td>
</tr>
<tr>
<td>12</td>
<td>5500-1235</td>
<td>Capillary ST 0.17 mm x 380 mm, long socket</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 x col - vlv, 6 x vlv - col</td>
</tr>
<tr>
<td>1</td>
<td>5063-6591</td>
<td>PEEK Fittings 10/PK</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Column outlet fitting, 12 x for cap. 5500-1235</td>
</tr>
<tr>
<td>1</td>
<td>5062-8541</td>
<td>Fingertight fitting long</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10/pk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Column outlet capillary valve 2 ports</td>
</tr>
<tr>
<td>1</td>
<td>5065-4454</td>
<td>Long Fittings and Ferrules 10/pk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 x for cap. 5500-1235 valve to column, 2 x for cap. 5500-1236</td>
</tr>
<tr>
<td>2</td>
<td>5067-4607</td>
<td>Capillary ST 0.17 mm x 280 mm SX/SX</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bypass line, TCC heater - vlv</td>
</tr>
<tr>
<td>2</td>
<td>0890-1713</td>
<td>Waste tubing, 2 m</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Waste-line</td>
</tr>
<tr>
<td>1</td>
<td>5067-1540</td>
<td>SST hex head nut with PEEK ferrule, 6/pk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Steel fitting with PEEK nut, 6 x for cap. 5500-1235 valve to column (column side)</td>
</tr>
<tr>
<td>1</td>
<td>5042-9918</td>
<td>Column clip set, 8 colors</td>
</tr>
<tr>
<td>1</td>
<td>5041-2115</td>
<td>Folding box</td>
</tr>
</tbody>
</table>
# Low Dispersion Capillary Kit for Long Columns

The components of the low dispersion capillary kit for long columns (Cap Kit 0.12 mm LDHE double 8/9 vlv long col (5067-4289)) are listed below.

Valve 1 refers to the inlet valve, and Valve 2 refers to the outlet valve.

<table>
<thead>
<tr>
<th>#</th>
<th>p/n</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>G1316-60005</td>
<td>LD-Pre-Column Heatexchanger Double-Assy</td>
</tr>
<tr>
<td>6</td>
<td>5500-1201</td>
<td>Capillary SST 0.12x105mm SL-- PS-LS \ Heat exchanger to column, SL fitting pre-swaged</td>
</tr>
<tr>
<td>6</td>
<td>G7167-68703</td>
<td>Fitting Intermediate Kit</td>
</tr>
<tr>
<td>1</td>
<td>G1316-90123</td>
<td>Technical Note *Installation of the Low Dispersion Heat-Exchanger Double Assemblies in the 1290 Series Thermostatted Column Compartment (G1316C), ENG*</td>
</tr>
<tr>
<td>1</td>
<td>5500-1191</td>
<td>Capillary ST 0.12 x 280 mm, long socket \ Valve 2 to detector</td>
</tr>
<tr>
<td>1</td>
<td>5067-4669</td>
<td>Capillary ST 0.12 mm x 600 mm S/SL \ Autosampler to valve in dual stack configuration</td>
</tr>
<tr>
<td>1</td>
<td>5067-4647</td>
<td>Capillary ST 0.12 mm x 340 mm S/SX \ Therm. ALS to valve</td>
</tr>
<tr>
<td>6</td>
<td>5500-1251</td>
<td>Capillary ST 0.12 mmX 400 mm SL/SL \ Valve 1 to heat exchangers (PL29)</td>
</tr>
<tr>
<td>6</td>
<td>5500-1192</td>
<td>Capillary ST 0.12 mm x 500 mm, long socket \ Columns to valve 2</td>
</tr>
<tr>
<td>1</td>
<td>5067-4607</td>
<td>Capillary ST 0.17 mm x 280 mm SX/SX \ Bypass line</td>
</tr>
<tr>
<td>1</td>
<td>5063-6591</td>
<td>PEEK Fittings 10/PK \ Column outlet fitting 2</td>
</tr>
<tr>
<td>1</td>
<td>5062-8541</td>
<td>Fingertight fitting long \ 10/pk \ Column outlet capillary valve 2 ports 4</td>
</tr>
<tr>
<td>1</td>
<td>5042-9918</td>
<td>Column clip set, eight colors</td>
</tr>
<tr>
<td>1</td>
<td>5041-2115</td>
<td>Folding box</td>
</tr>
<tr>
<td>2</td>
<td>0890-1713</td>
<td>Waste tubing, 2 m \ Waste-line</td>
</tr>
</tbody>
</table>
3 System Setup and Installation

Documentation of the Individual Modules 34
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Configurations and Capillary Setup 38
   Configuration and Capillary Setup (G7116B) 38
   Setup Examples 40
Recommended Column Configurations 42
Installing the Solvent Selection Part 44
   Deciding on the Position of the Degasser 45
   Installing the Solvent Tubing 47

This chapter provides information on system setup, installation of valve heads, heat exchangers and capillaries, and installation of solvent selection parts.
The MDS works in lots of different hardware configurations. For details of the individual modules mentioned in this guide, refer to the Agilent Information Center (AIC).
System Setup

The MDS supports lots of different hardware configurations. Examples for different proved and tested method development system set-ups are shown in Figure 6 on page 35, Figure 7 on page 36, and Figure 8 on page 37.

![Diagram of MDS setup]

**Figure 6** Example setup based on a 1290 Infinity II LC
3 System Setup and Installation

System Setup

Figure 7 Example setup based on an Agilent 1260 Infinity Multisampler with Quaternary Pump G1311B
In principle, the connecting capillaries should be kept as short as possible to reduce extra-column band-broadening, and to keep the backpressure small. It is very important to keep the distance to the detector as short as possible. The next important connection is from the autosampler to the column compartment. Several set-ups taking these considerations into account are covered with the available capillary kits.

**Figure 8** Example setup based on a 1290 Infinity II LC (with two MCT), two stack configuration
Configurations and Capillary Setup

Configuration and Capillary Setup (G7116B)

**CAUTION**

Damage to the rotor seal

Instant pressure release within the valve will lead to water jet effects that can harm internal parts of the valve. This pressure release typically happens if the valve gets switched under high pressure over unused or open channels.

➔ Block all unused channels properly with the M4 blank nut.

**NOTE**

To minimize valve movement over open connections it is recommended to plumb the column connected channels in one row.

e.g.:

- channel 1 – column 1
- channel 2 – column 2
- channel 3 – column 3
- channel 4 – column 4
- channel 5 – blocked
- channel 6 – blocked
- channel 7 – waste
- channel 8 – bypass

**NOTE**

The blank nuts are only required for the ports on the inner circle that connect the valve with the column inlet.
1 Install the in and out connectors.
   - from sampler to the valve (Capillary ST 0.12 mm x 500 mm M4-SL PS-PS (5500-1202))
   - from valve to the detector (Capillary ST 0.12 mm x 280 mm M4-SL PS-PS (5500-1203))

![Image of a valve with In and Out ports]

The In port is hydraulically connected to the column inlet ports 1-8 on the inner ring while the Out port connects to the column outlet ports 1¨-8¨ on the outer ring.

2 Install the column inlet and outlet connections.

<table>
<thead>
<tr>
<th>Connections</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ports 1-8</strong></td>
<td>for connections from valve to the heat exchanger (Capillary ST 0.12 mm x 130 mm M4-SL PS-PS (5500-1199)) or waste line (Waste tube (G1375-87326))</td>
</tr>
<tr>
<td><strong>ports 1¨-8¨</strong></td>
<td>for connections from column outlet to valve (Capillary ST 0.12 mm x 130 mm SL/M (5500-1200)), use fingertight PEEK fittings for connecting the column outlet</td>
</tr>
</tbody>
</table>

![Image of a column outlet fitting with From column and To heat exchanger labels]
Setup Examples

1. 8 Column-selection

**Figure 9** Hydraulic flow path schematics for an 8-column selection setup
2 Six column selection with purge line and valve-bypass

Figure 10 Hydraulic flow path schematics for 6-column selection setup with purge and valve bypass line.
Recommended Column Configurations

Column Configurations with one MCT

The column configurations with the MCT are very flexible and cover most of the major requirements for method development. MCT configurations with a Column Selector Valve and Quick Connect Heat Exchangers offer the following options:

- Quick change between up to eight (G7116B) respectively four (G7116A) different columns of 100 mm length
- Different stationary phases for different applications, or
- Identical stationary phases in columns with different dimensions for either faster run-times or higher resolutions, or
- Different internal diameters for loading studies
- Individual setting of temperature for each column

Figure 11  The G7116B 1290 Infinity II Series Multiple Column Thermostat equipped with a Quick-Change 8 Column Selector Valve
Column Configurations with two MCT (for long columns)

The same situation as described in “Column Configurations with one MCT” on page 42 can be achieved using two MCT modules and one valve. In this configuration it is possible to use up to 8 long columns (or several combinations with short columns). If more columns are needed, see “A Valve Thermostat Cluster (VTC) for up to 32 Columns” on page 27.

NOTE
Agilent 1260 Infinity II Multicolumn Thermostat (G7116A) does not support clustering!
Installing the Solvent Selection Part

Especially for LC method development, but also for frequently changing application needs, it is very important to have a large choice of different mobile phases available because the pH value, the organic modifier, and the ionic strength have a large influence on the separation. Therefore, the Agilent 1200 Infinity Series Multi-method and Method Development Systems offers the possibility to equip the pump with up to two additional external valve drives (G1170A), equipped with a 12-position/13-port solvent selection valve head (G4235A). This multiplexes a channel of the pump and provides up to 12 additional solvent channels. With the quaternary pump, this gives a maximum of 26 available solvents and allows the generation of up to 169 solvent combinations (for a binary gradient), or 288 unique solvent combinations (for ternary gradients). When the external solvent selection valves are combined with a binary pump with no internal solvent selection valve, 144 solvent combinations are possible. If the binary pump is fitted with an internal solvent selection valve, providing two different solvents per channel, up to 169 solvent combinations (for a binary gradient) are possible. For an overview, see Table 2 on page 44.

Table 2  Possible Solvents and Solvent Combinations in Different Configurations

<table>
<thead>
<tr>
<th>Available Solvents:</th>
<th>0</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1312B</td>
<td>2</td>
<td>13</td>
<td>24</td>
</tr>
<tr>
<td>G4220AB, G7120A, G1312B (SSV installed)</td>
<td>4</td>
<td>15</td>
<td>26</td>
</tr>
<tr>
<td>G1311B, G4204AB, G7104A</td>
<td>4</td>
<td>15</td>
<td>26</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Binary Solvent Combinations:</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>G1312B</td>
<td>1</td>
<td>12</td>
<td>144</td>
</tr>
<tr>
<td>G4220AB, G7120A, G1312B (SSV installed)</td>
<td>4</td>
<td>26</td>
<td>169</td>
</tr>
<tr>
<td>G1311B, G4204AB, G7104A</td>
<td>4</td>
<td>36</td>
<td>169</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ternary Solvent Combinations:</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>G1311B, G4204AB, G7104A</td>
<td>4</td>
<td>36</td>
<td>288</td>
</tr>
</tbody>
</table>
Deciding on the Position of the Degasser

It is recommended to use the built-in degassing unit, and to place it in the flow path behind the external solvent selection valve. The built-in degassing unit has a low delay volume and a very high degassing efficiency.

The Agilent 1260 Infinity Binary Pump (G1312B) does not have a built-in degasser. For Agilent 1260 Infinity Binary LCs, you need to decide where in the flow path to position the external degassing unit. There are two options available: Solvent degassing can be done before or after the external solvent selection valve.

If two external solvent selection valves are configured in the system, only degassing either before or after the external solvent selection valve is supported. A configuration with one valve before the degasser and one after it is not supported.

Degassing before the solvent selection valve

Pro: Short flush times (the degasser chamber does not need to be flushed)

Con: Potential re-solution of air in the longer tubing from the degasser to the pump, more than one degasser required

Required parts:

- the requisite number of G4225A degasser units (one degasser is for four solvents only)
- Solvent cabinet 1200 Infinity, including all plastic parts (5065-9981) (one is included to the pump)
- Solvent bottle, transparent (9301-1420) or
- Solvent bottle, amber (9301-1450)
- n x Solvent selection tubing kit, 4 solvents (5067-4601)
Degassing after the solvent selection valve

**Figure 13** Solvent degassing after the solvent selection valve

Pro: No risk of re-solution of air, only one degasser required

Con: Considerably longer flushing times because after every solvent change, the degasser chamber (with the G4225A ca. 1.2 mL) needs to be flushed thoroughly, which means with at least five times the volume of the degasser chamber.

Required parts:
- 1x G4225A degasser
- Solvent cabinet 1200 Infinity, including all plastic parts (5065-9981) (one is included to the pump)
- Solvent bottle, transparent (9301-1420) or
- Solvent bottle, amber (9301-1450)
- n x Solvent selection tubing kit, 4 solvents (5067-4601)
Installing the Solvent Tubing

One solvent selection tubing kit (Solvent selection tubing kit, 4 solvents (5067-4601)) is delivered with all required parts to connect up to four solvent bottles to the 12-Position/13-Port valve (G4235A) on the G1170A External Valve Drive. If you need more solvents, additional kits need to be ordered.

Depending on whether degassing is done before or after solvent selection, there are two possible set-ups for the hydraulic connections, as described below.

**Degassing with several degassers before the solvent selection valve:**

1. Place the solvent bottles in additional solvent cabinets on top of the degasser.
2. Use the pre-installed bottle head assemblies to connect the bottles with the degasser inlet ports.
3. Cut appropriate lengths of the 2 x 5 m solvent tubing and fit them to the fittings.

**NOTE**

Use the prefitted bottle head assemblies as examples of how these are fitted together.

4. Attach the PEEK-adapters to the ports of the 12-position/13-port valve and connect the solvent tubing from the outlet port of the degasser to the peripheral ports of the valve.
5. Connect the central port of the 12-position/13-port valve to one port of the pump, or to one port of the optional internal solvent selection valve of the pump (binary pumps only).
Degassing after the solvent selection valve:

The pre-installed tubings need to be replaced with much longer ones that have to be cut to appropriate lengths from the 5 m tubing.

1. Disassemble the bottle head assemblies.
2. Cut appropriate lengths of the 2 x 5 m solvent tubing.
3. Refit the bottle head assemblies with the longer tubings.
4. Connect the tubings with the peripheral ports of the 12-position/13-port valve using the PEEK adapters.
5. Connect the central port of the 12-position/13-port valve with the inlet port of one degasser channel and the corresponding outlet port with one channel of the pump.

In both cases, make the corresponding settings in the software (entering the clustering information, and entering the names of the solvents into the solvent table).
4

Configuring the System in ChemStation and Creating Methods

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Column Compartment Cluster Configuration (VTC) 51
Solvent Delivery Cluster Configuration 56
Settings for Column Compartments 59
Settings 60
Settings for Solvent Selection 64

This chapter explains how to configure the system in the control software and how to create methods.
Clustering of Modules in ChemStation using the RC.NET Drivers

During autoconfiguration, if the system detects that there are modules that can be clustered, a dialog box is displayed (Figure 14 on page 50) that allows you to select the modules via check boxes to be included in the cluster. You select the modules for the Column Compartment Cluster and the Pump/Valve Cluster separately.

![Screen Agilent LC Modules and Systems Auto Configuration](image)

**Figure 14**  Screen Agilent LC Modules and Systems Auto Configuration, Selection of modules to be included in the cluster

To start the clustering, klick one of the available cluster option buttons.
Column Compartment Cluster Configuration (VTC)

When you click **Configure Valve Thermostat Cluster** in the cluster creation dialog box (Figure 14 on page 50), the VTC configuration dialog box is displayed that allows you to configure the column compartment cluster.

Select the topology with the drop-down list and the topology scheme will be shown beneath. Red caution icons indicate, that valves are not defined yet.

**Example 1: Topology 1 VC for long columns**

Topology 1 enables clustering of one valve with up to eight columns. And with two MCT modules it is possible to use up to 8 long columns.

![Valve Thermostat Cluster Post Auto Configuration](Image)

*Figure 15* Screen **Valve Thermostat Cluster Post Auto Configuration**, Configuring Topology 1
First you have to update your module list by adding or removing modules. Use the up/down arrow buttons to move the clustered column compartments up or down in the table. The position in the table should reflect the physical set-up of the system. The sequence you select here is displayed in all column compartment cluster images.

Click **Configure...** (when you add a module, you are automatically asked to configure it after having confirmed your module type) and select your valve version by a drop-down list with a click on **Not installed** (see Figure 16 on page 52).

![Communication Options](image)

**Figure 16**  **Screen Configure single device**, installing valve versions

If this setting is completed, you have to assign the valve in the topology scheme with a click on the red caution icons or finger icons. A context menu will show available valves. This needs to be repeated for each valve in the topology.

The Plumbing section enables you to specify how the installed columns are connected to the column selection valve, and where they are located in the column compartment cluster. The valve positions are color-coded; the colors are reflected in the Column Assignment diagram (see Figure 23 on page 60).

Colors usages and locations are all specified using drop-down menus in the relevant cells. Column locations are specified by column compartments (**Column Host**) and position (**Location**). Note that if you specify a long column occupying both left and right positions in a column compartment, the temperatures of both heat exchangers are coupled and set to the same temperature. This has implications for any other columns in the same column compartment. Any conflict in the configuration is marked with a red warning sign, and a tooltip gives the reason for the error.
For Plumbing, select the Color Code, Usage, Column Host and Location from the drop-down list which appears by clicking on the corresponding table cell.

With this flexible system, all selectable plumbings are possible. But to keep track of the system, a logical assignment is very helpful. You can see all paths in the topology scheme by clicking on the path number in the plumbing table. Finally you will have an overview of the set hydraulic connections by clicking Print Connections....

Example 2: Topology 2 $V(VC)_2$

With branched topologies it is possible to increase the number of columns. In topology 2, the simplest form, typically an external root valve (first level) branches between two MCTs (second level).

Figure 17 Screen Valve Thermostat Cluster Post Auto Configuration, Configuring Topology 2
When the valves are assigned (see Figure 15 on page 51), in branched topologies a default port of the host valve (level 1) is proposed (indicated by a number left to the valve). This is the port of the valve to the left (level 1 valve), where the valve to the right (level 2 valve) is connected to. In order to change this port (e.g. to achieve a shorter capillary connection), click on the number and select a different port number from the drop-down list (see Figure 18 on page 54).

**NOTE**
The selected number is the valve switching position and not the port (they may be equal).

**Figure 18**  Valve switching positions

Topologies 3 and 4 are configured in the same way.
Example 3: Topology 5 V-C-V

This topology uses two bracketing valves: one for inlet and one for outlet. The left valve is the inlet valve in the high pressure flow path, the right one the outlet valve in the low pressure flow path downstream to columns. With this topology TCC-clustering is possible and can only be used with two 8-pos/9-port valves.

Figure 19  Screen Valve Thermostat Cluster Post Auto Configuration, Configuring Topology 5
Solvent Delivery Cluster Configuration

When you click **Configure Pump/Valve Cluster** in the cluster creation dialog box (Figure 14 on page 50), the **Pump Valve Cluster Configuration** dialog box is displayed that allows you to configure the solvent delivery cluster (see Figure 20 on page 56).

\[
\text{Figure 20} \quad \text{Screen Pump Valve Cluster Configuration, Tab Configuration}
\]

During autoconfiguration, the dialog box sections contain the pump and valve(s) that were selected in the cluster creation dialog box. To complete the configuration of the solvent delivery cluster, you specify the pump channels the valves are associated with by selecting from a drop-down list in the **Channel Configuration** table. The table lists all available channels, depending on the configured pump:

- **Quaternary pump**: A, B, C, D
- **Binary pump**: A, B
- **Binary pump with internal solvent selection valve**: A1, A2, B1, B2
**Solvent configuration**

Use the **Solvents** tab, available in the post-autoconfiguration mode, to specify the solvent for each column. All available channels are listed; the number of available channels depends on the configured pump and the number of valves in the cluster.

In addition to specifying the solvent name and either specifying a compressibility value (G1311B, G7111B) or selecting a solvent definition (G1312B, G4220A/B, G7120A), you can optionally specify other solvent characteristics (pH, viscosity and molarity).

![Figure 21 Screen Pump Valve Cluster Configuration, Tab Solvents](image)

**Figure 21**  Screen **Pump Valve Cluster Configuration, Tab Solvents**

**Configure Solvent Type Catalogs** opens the **Solvent Type Catalogs**, which lists all available solvent types.
The solvent name that is reported is automatically concatenated from your entries in the columns **Solvent**, **pH** and **Molarity** (if you enter values for pH and/or molarity) as in the following example:

<table>
<thead>
<tr>
<th>Column</th>
<th>Your entry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent</td>
<td>Phosphate buffer</td>
</tr>
<tr>
<td>pH</td>
<td>4.5</td>
</tr>
<tr>
<td>Molarity</td>
<td>20</td>
</tr>
</tbody>
</table>

The **Solvent Name** automatically generated and used by the system will be:  
*Phosphatebuffer pH: 4.5 20mM*

**NOTE**
The pH value is used by the Method Scouting Wizard to check for potential incompatibilities of columns and solvents. The viscosity column is for future use.

**Bottle fillings**

Bottle sizes and fillings are managed by the standard bottle filling interface. The system automatically assigns the values to the selected solvents of the external valves according to the valve positions.
Settings for Column Compartments

Columns definition

To specify the columns, select Instrument (Agilent 1100/1200 System) > Columns. Fill in the columns you regularly use in your lab, see Figure 22 on page 59. This information is required for configuring the column locations shown in Figure 23 on page 60. You should enter at least a Description, the physical dimensions and, if these values are identical for some columns, a serial number to allow correct identification later. If you are using the Method Scouting Wizard, it is important that you set the column void volume correctly using [mL] as units, otherwise flushing and equilibration times will not be calculated correctly.

Alternatively, the total porosity can be entered by selecting [%] as units (e.g. 60 %). In this case the column dimensions in [mm] must be entered as well otherwise the column void volume cannot be calculated.

When you are using the Method Scouting Wizard, it is also advisable to enter a maximum temperature and maximum and minimum pH values. It is not required to enter the Installed value (this entry is needed only for non-method-development systems). See in the User Contributed Libraries on the ChemStation DVD for a tool to import column tables.

![Table of columns which are used for method development experiments](image)

**Figure 22** Screen Edit Columns. Table of columns which are used for method development experiments

The Installed value is not used in case of a column compartment cluster.

Plumbing... leads you directly to the Column Assignment (see Figure 23 on page 60).
Settings

**Plumbing** (see Figure 23 on page 60) enables you to specify how the installed columns are connected to the column selection valve, and where they are located in the Thermostat.

The valve positions are color-coded; the colors are reflected in the **Column Tag Information** and shown in the **Visualization** scheme.

![Figure 23 Screen Column Assignment](image)

The **Column Tag Information** tab shows information about the types of columns used for analysis. With a click on the icons in the **Import** column, you can select the column from a list which can be imported with all parameters (see Figure 24 on page 61).
Configuring the System in ChemStation and Creating Methods

Settings for Column Compartments

Figure 24  Screen Import Column From Column Database

Method setup for Multicolumn Thermostat

After configuration of all columns, select Instruments > Set Up Column comp. (G7116B) to setup the parameters for analysis.
4 Configuring the System in ChemStation and Creating Methods

Settings for Column Compartments

Figure 25 Screen Method of VTC, Setting parameters for the analytical run (MCT)
The user interface allows to set the following parameters:

- **Path/Column**
  The position of the column in the MCT and the corresponding valve position are shown in the graphical representation. Together with the color code, this allows the identification of the flow path.

  The column used for the individual method can be selected in the Method window of the 1290 Infinity II Multicolumn Thermostat. The appropriate column can be selected either by the drop-down menu, which shows all assigned columns, or by just clicking the column with the correct color code in the image of the 1290 Infinity II Multicolumn Thermostat. The current valve position, which automatically connects to the chosen column, is shown. For quick information, the valve position, the color code of the chosen column, and its product number are shown in Agilent ChemStation.

- **Temperature of Selected Zone**
- **Stop-time**
- **Posttime**
- **Advanced**
  In **Temperature Settings**, a list of the temperature zones is given and the corresponding options for enabling analysis. Selected columns in the schema are indicated in the list with a blue arrow.

  The software recognizes the position of the front door (open or closed) of the MCT. By default, an analysis starts only when the door is closed (recommended); however, when the corresponding check box is marked, the analysis is enabled even with an open door.

- **Timetable**
  If you need to apply temperature gradients during a run, click **Add** and enter the additional time programming of the temperature.
Settings for Solvent Selection

Configuring the solvent table

If an external G4235A solvent selection valve is clustered, solvents can be specified and named by selecting Instrument > More Pump Valve Cluster > Pump Valve Cluster Configuration, see Figure 26 on page 64.

![Screen Pump Valve Cluster Configuration, Tab Solvents](Image)

The left column shows the valve position. In the Solvent Name column, you can assign a name to the solvent attached to that valve position. For pumps using calibration tables for the compressibility and elasticity of the solvents (for example the G1312B Binary Pump), you also need to enter the Calibrated Solvent here. The entry for the pH Value is optional, but is recognized in the Method Scouting Wizard to exclude incompatible combinations of solvents and columns.
Actual Volume and Total Volume are set in the Bottle Fillings dialog (also for the solvents attached to the solvent selection valve), see Figure 27 on page 65.

![Figure 27 Screen Bottle Fillings](image-url)
Method setup for solvent delivery cluster

In the pump setup screen, the solvents for an experiment can be selected without the need to switch the solvent selection valve to the appropriate position in an additional screen, see Figure 28 on page 66. You simply select the solvent that you want to use by its name. If you need to enter a calibrated solvent for the pump that is installed in your system, it is taken automatically from the solvent table (see Figure 26 on page 64).

![Figure 28 Screen Method of PumpValveCluster, Selecting a mobile phase attached to the solvent selecting valve](image)

After configuring and setting up the complete system, you can set up the different methods for the different columns, set up the sequence and start it.
This chapter provides information on installation, use and features of the software.
Overview of the Agilent Method Scouting Wizard

The Agilent ChemStation Method Scouting Wizard is a software add-on to the OpenLAB CDS ChemStation that enables you to set up methods and sequences for easy and logical method scouting using an Agilent Method Development System. It allows you to create all methods and a sequence that have all possible combinations of available columns, solvents, a set of predefined gradients and a set of predefined temperatures.

The Method Scouting Wizard automatically generates all steps to flush the system with any required solvents, performs column equilibration procedures, and can store columns in predefined storage solvents. In this respect, it uses waste and/or available bypass lines intelligently to allow fast flushing procedures. Flushing, equilibration and column storage procedures, and temperature changes are arranged in the workflow such that a minimal number of these steps need to be performed to save valuable time and solvents. In Figure 29 on page 68, a part of a complete sequence is shown as an example of how the different steps are put together.

**Figure 29** Different steps during a method scouting campaign as generated by the Agilent ChemStation Method Scouting Wizard
The analysis is performed on column 1 using a solvent combination A/B, followed by post-run storage of column 1 in a special storage solvent D, and finally switching to column 2 using a new solvent combination A/C. The system has a waste line available (see “Specifying and Selecting Gradients” on page 80 and “Settings for System Volumes, Flushing, Equilibration and Column Storage” on page 88).
Features

The Agilent Method Scouting Wizard provides customer benefit in LC method development. For an overview on features of the software, see Table 3 on page 70.

Table 3  Features of Agilent Method Scouting Wizard

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Version</td>
<td>MSW A.02.07</td>
</tr>
<tr>
<td>Sample introduction</td>
<td>Any of the Agilent 1200 Infinity Series Autosamplers supported by LC&amp;CE Drivers A.02.16 can be used. Select your autosampler suitable to the required maximum pressure capability of the system. In particular, the following sampler is introduced: • 1260 Infinity II SFC MultiSampler (G4767A)</td>
</tr>
</tbody>
</table>
| Supported screening pumps            | • 1290 Infinity Binary Pump (G4220A)  
• 1290 Infinity Binary Pump VL (G4220B)  
• 1290 Infinity Quaternary Pump (G4204A)  
• 1260 Infinity Quaternary Pump (G1311B)  
• 1260 Infinity Binary Pump (G1312B)  
• 1260 Infinity Bio-inert Quaternary Pump (G5611A)  
• 1260 Infinity SFC Binary Pump (G4302A)  
• 1290 Infinity II High Speed Pump (G7120A)  
• 1290 Infinity II Flexible Pump (G7104A)  
• 1260 Infinity II Quaternary Pump (G7111B)  
• 1260 Infinity II Quaternary VL Pump (G7111A)  
• 1260 Infinity II Binary SFC Pump (G4782A)  |
| Support of pump clusters             | • Pump Valve Cluster with all pumps above, and with •  G1170A  
•  G4235A  
•  1260 Infinity Preparative Pump Cluster (2x G1361A)  |
| Support of multiple screening pumps, as e.g. in SFC hybrid system | If more than one pump is configured, as e.g. with the G4302A plus LC pump, the user can select which pump will be used in the screening campaign.  |
| Pump specific configuration parameter | The default parameter used for flushing, equilibration, and column storage can be stored separately for each pump.  |
The Agilent ChemStation Method Scouting Wizard
Overview of the Agilent Method Scouting Wizard

Table 3  Features of Agilent Method Scouting Wizard

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supported column screening configuration</td>
<td>• 1260 Infinity II Multicolumn Thermostat (G7116A) with 4/10 valve&lt;br&gt;• Single 1290 Infinity II Multicolumn Thermostat (G7116B) with 4/10, 6/14 or 8/18 valve&lt;br&gt;• Valve Thermostat Cluster solution for using up to 32 columns per system.&lt;br&gt;  • Multiple valve hosts (Flexible Cubes (G4227A) are not yet supported!)&lt;br&gt;   • 1290 Infinity II Multicolumn Thermostat (G7116B)&lt;br&gt;  • 1290 Infinity Valve Drive (G1170A)&lt;br&gt;  • 1290 Infinity Thermostatted Column compartment (G1316C)&lt;br&gt;  • Temperature zones&lt;br&gt;   • 1290 Infinity II Multicolumn Thermostat (G7116B)&lt;br&gt;   • 1290 Infinity II Integrated Column Compartment (G7130A)&lt;br&gt;   • 1290 Infinity Thermostatted Column compartment (G1316C)&lt;br&gt;   • 1260 Infinity Thermostatted Column compartment (G1316A)&lt;br&gt;  • Column compartment cluster of 2 or 3 1290 Infinity Thermostatted Column Compartments (G1316C)</td>
</tr>
<tr>
<td>Report templates – Intelligent Reporting</td>
<td>• Several report templates are provided to support identification of most interesting chromatograms.&lt;br&gt;• Special report template available dedicated for export to a spread sheet program, e.g. MS EXCEL.&lt;br&gt;• “Interactive” Report templates where you can set custom criteria interactively to filter your result set. Filter are provided for criteria like Number of Peaks, Minimum Resolution etc.</td>
</tr>
<tr>
<td>Automatic scheduling of scouting sequences</td>
<td>The system will automatically schedule a screening sequence (OpenLAB ChemStation).</td>
</tr>
</tbody>
</table>
The Agilent ChemStation Method Scouting Wizard
Overview of the Agilent Method Scouting Wizard

Table 3  Features of Agilent Method Scouting Wizard

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scouting Sequence Optimization</td>
<td>MSW decides on the optimal sequence run order now based on calculating <em>several</em> different run orders with varying combination priorities of solvent, column and temperature changes. These single results, by itself optimal in their run structure, are finally compared to determine the overall optimal sequence with the shortest total run time and best solvent usage under given setup conditions.</td>
</tr>
<tr>
<td>Support Intelligent System Emulation Technology (ISET)</td>
<td>MSW supports ISET functionality with solvent screening when using a pump method with enabled emulation mode (ISET)</td>
</tr>
</tbody>
</table>

Software Compatibility

To take full advantage of Agilent 1290 Infinity II Method Development Solution, Agilent Method Scouting Wizard (MSW) software is recommended.

To run MSW A.02.07 with all its features, it is required to have OpenLAB CDS ChemStation Edition C.01.07 SR3 installed.

This MSW version is not backwards compatible and cannot be installed on a lower ChemStation revision.

Please be aware, that neither MassHunter nor EZChrom support MSW.

Instrument Driver

The MSW only supports LC RC.Net drivers; the classic driver architecture is NOT supported!

The following minimal LC RC.Net driver is required for revision MSW A.02.07:
- Agilent LC/CE Driver Package A.02.16

Make sure that driver version A.02.16 is installed:
- Check the installed driver version with your OpenLAB CDS ChemStation Edition C.01.07 SR3.
- Upgrade or downgrade if the driver version is different.
Software Installation

**Prerequisites**

If there is a previous version of the Method Scouting Wizard on your computer, open the Windows Control Panel, select **Add or Remove Programs** and select to remove the previous version.

1. To install the Agilent Method Scouting Wizard, follow the instructions given in the Install Instructions.pdf located on the Method Scouting Wizard disk.
Defining the Campaign

All methods, the sequence, and the project file that contains all settings of a campaign are saved in a screening-campaign folder. You can create a new campaign or open an existing one. In the latter case, you can choose between overwriting the old one and saving it with a new name. Create a new screening campaign includes naming of the campaign and specifying the path where the campaign is saved. It is recommended to save the campaign in a separate folder, such as Screening. This screen is always the start for the setup of an experiment (see Figure 30 on page 74).

NOTE

You can open a screening campaign from the previous revision of Method Scouting Wizard. If the current instrument configuration does not match the instrument configuration of the old campaign, the campaign will be adjusted accordingly. When you save the campaign, it is automatically converted into the format of the current revision.
You can also view an existing screening campaign; all the information for the campaign is displayed in the sequence of screens, but you cannot make any modifications, and all buttons that allow modification are hidden. The compatibility of the current instrument configuration is not checked, and the base method is not required to be available. This allows you to review the content of any existing campaign without modifying it.
Define Screening Campaign Base

For a method scouting campaign, a base method needs to be set up (you do this as usual in the ChemStation - it is recommended to use Edit Entire Method). All parameters that are not changed are taken from this base method. These are typically the detector settings, autosampler settings, pump settings that are not altered, and data processing parameters (see Figure 31 on page 76).

In addition to selecting the base method, you also need to specify the scope of the screening campaign. That means the combinations of column screening, solvent screening, gradient screening and temperature screening that you would like to perform. Here, you specify the dimensions of the method scouting campaign; in the next screens, the ranges of the selected dimensions are specified.

**NOTE**

With a quaternary pump ambiguities could occur if "solvent screening" is selected and the gradient would be taken form the base method during solvent screening. To prevent this if no gradient screening is selected, the "gradient screening" will be checked automatically. If no gradient screening is demanded by the user only one gradient has to be defined in the later gradient screening page.

![Figure 31](image.png)  
*Screen for defining the campaign range and selection of the master method*
Selecting the Columns

In this screen, you select the columns to be evaluated (see Figure 32 on page 77). You can select only the columns that are installed in the system and have been specified previously in the Configuring Columns user interface screen under Instruments. The installed columns as defined will be shown in the Method Scouting Wizard; important variables such as the position, the specified void volume, the maximum temperature and pH-value are shown, but are not editable. Column properties are read from the Columns table of the ChemStation. You select the columns you want to use in the method scouting campaign by checking the Use check boxes.

Figure 32  Selection of columns

The two check boxes below the table allow you to scale flow rates and gradients for columns with different dimensions.

Scale flow for column with largest diameter ensures that the velocity of the eluent is maintained irrespective of the diameter of the column. Flow rates of narrower columns are reduced relative to those of wider columns in the ratio of the squares of the column internal diameters.

Scale (gradient) run times for longest column ensures that the full gradient will run on all columns, irrespective of their length. All gradient time points, the run time and the posttime for shorter columns are reduced relative to those of longer columns in the ratio of the column lengths.

If you do not select column screening as a dimension of the method scouting campaign, the column specified in the base method is used.
Selecting the Solvents

If solvent screening is selected, the solvents to be varied are specified in the following screen (see Figure 33 on page 78). The Method Scouting Wizard automatically detects the system configuration and shows the appropriate screen. For a binary pump without any additional solvent selection valves, no solvent choices are available. If an additional internal solvent selection valve is installed, the solvents to be used can be selected. If an additional external G4235A solvent selection valve is installed, all solvents that are available and specified in the solvent table are shown as choices for the corresponding channel where the valve is installed. You mark the corresponding check boxes of the solvents that are used to create binary gradients.

Fill in the ratio of channel A to channel B (in %).

![Figure 33](image)

If a quaternary pump is installed, the Set Up Solvent Screening screen looks slightly different, see Figure 34 on page 79. Again, the position of an optionally installed external solvent selection valve is detected. Additionally, you choose
to create binary, ternary or quaternary gradients. Besides, for quaternary gradients that do not allow further choices you specify here which variations should be created, that is for a binary gradient on a system with an external solvent selection valve attached to channel A, for example, combinations of those solvents on A versus the B-, C- and D-channel (A_01-B, A_01-C, A_01-D, A_02-B, A_02-C, A_02-D, etc). Other combinations might be solvents on the A-channel as well as the B-channel versus the C- and D-channel (A_01-C, A_01-D, A_02-C, A_02-D, B-C, B-D, etc).

The number of selected solvent combinations and the maximum possible number of combinations with the given system are indicated at the bottom of this screen.

Figure 34  Set up solvents screening using a quaternary pump, a setup for a binary gradient of channel A (with an attached external solvent selection valve) versus the channels B, C and D is shown.

If no Pump Valve Cluster is set-up and you use a single pump, the entry of the pH value is not supported at the driver level. In this case, the Agilent ChemStation Method Scouting Wizard allows you to enter the pH-values of the solvents at this point.
Specifying and Selecting Gradients

If the gradient is also to be varied, the following screen is displayed, where you can specify gradients, their initial composition, run time, post-time and the flow rate to be used. You have the possibility to enter the gradients based on a table (time vs. percentage of solvent, see Figure 35 on page 80) in the Table tab or graphically (see Figure 36 on page 81) in the Graph tab. With the graphical tool, you can set gridlines and specify that the cursor snaps onto the gridlines, with selectable snapping ranges. Finally, you can also overlay the selected gradients in the Overlay tab, see Figure 37 on page 81.

Figure 35  Defining and selecting different gradients - gradient table
The Agilent ChemStation Method Scouting Wizard
Specifying and Selecting Gradients

Figure 36  Defining and selecting different gradients – graphical interface

Figure 37  Defining and selecting different gradients – overlay interface
It is extremely important to set the initial composition correctly. This is the composition that the pump generates after the method is loaded but the injection has not yet occurred, and the composition that is used after the run time (data acquisition) has elapsed and until the next method is loaded (for example, during the post-time or system overhead time). If you do not explicitly change the initial composition the one from the base method is used.

The initial composition determines the settings for a flushing or equilibration method that is executed immediately before the gradient method is applied to a sample, or a subsequent column storage method that does not use a dedicated storage solvent.

A simple linear gradient consists of one line in the table – the final composition that has to be reached. If the time value of the final composition is lower than the run time, the final composition is held until the run time is reached. If you have set a post-time, the initial composition is applied from the “run time” until the “post-time” (see Figure 38 on page 82). To correctly specify gradients, you also need to consider how the Method Scouting Wizard applies the Equilibration procedures (see “Settings for System Volumes, Flushing, Equilibration and Column Storage” on page 88). Take care when setting time values at 0 min or close to 0 min; unless this value matches the initial composition, you will have a situation with an improperly equilibrated column and maybe non-reproducible results, see Figure 40 on page 83).

**NOTE**
Be sure to use either the post-time or to have a suitable time point set in your gradient method to ensure column equilibration after repeated use of the analytical method (e.g. multiple injections or multiple samples) See “Settings for System Volumes, Flushing, Equilibration and Column Storage” on page 88.
The Agilent ChemStation Method Scouting Wizard
Specifying and Selecting Gradients

**Figure 39** Typical gradient setting without post-time but an added equilibration period in the gradient table. Data acquisition occurs during the equilibration phase of the column.

**Figure 40** Avoid composition settings at 0 min or close to 0 minutes in the gradient table that do not match the composition of the initial composition as this leads to improper equilibration.

**NOTE**

The pump flow rates apply at least for the methods for columns with the largest internal diameter. If the Scale flow for column with largest diameter check box is marked on the Set up column screening page, then the flow rates will be adjusted relative to the squares of the column IDs.

The gradient times apply at least for the methods for the longest columns. If the Scale (gradient) run times for largest column check box is marked on the Set up column screening page, then the gradient times will be adjusted relative to the column lengths.
Defining and Selecting the Temperatures

If you also selected temperature screening, you can enter the temperatures you want to test (see Figure 41 on page 85). You can enter all values that are available for the given system.

NOTE

If you are using a mixed system containing a G1316C and a G1316A column compartment, the maximum temperature is defined by the G1316A (80 °C instead of 100 °C). The lowest temperature that can be entered is -5 °C.

The G1316-series column compartments are able to cool to 10 °C below ambient, but since the ambient temperature can change during the course of the analysis series, you should consider having some safety margin. If your lab has a rather high ambient temperature, you might want to set the lowest screening temperature to approximately 20 – 25 °C. If this temperature cannot be reached, the column compartments stays in a not-ready state because the Method Scouting Wizard enforces the analysis to be enabled only when the column compartment has reached the set-point in the allowed range as specified in the base method (Set Up Column Thermostat Cluster > Enable Analysis). For flushing or column storage methods, the method starts without reaching the set point.
Defining and Selecting the Temperatures

Figure 41  Temperature screening
Review the Selected Methods

This screen shows you an overview of all combinations of columns, solvents, gradients and temperatures (see Figure 42 on page 87). Additionally, two check boxes at the bottom allow you to automatically deselect incompatible combinations of columns with solvent-pH and temperatures. This option requires that the values for max temp. and max/min pH are set for the columns in the ChemStation column database, and that the solvent pH is set correctly in the solvent table of the pump if an external G4235A solvent selection valve is clustered to the system or in the solvent screening interface. The allowed pH range and temperature of the column is shown in the table and marked either with a green or red color to indicate compatible and incompatible values. If the maximum temperature value for a column is not available, you cannot exclude columns on the basis of temperature; if the pH values for a column are not available, you cannot exclude columns on the basis of pH.

By clearing the Use check boxes in the left column of the table, you can eliminate unwanted combinations of the full matrix. This is very useful, because it allows you to save and re-use such special scouting campaigns with other samples.

The total number of methods is shown at the bottom, and gives a quick overview on the complexity of the specified scouting campaign. Detailed information is available at the end after flushing, column storage and equilibration procedures have been specified and added to the campaign.
Review the Selected Methods

Figure 42  Review and select analytical runs to be performed
The Agilent ChemStation Method Scouting Wizard
Settings for System Volumes, Flushing, Equilibration and Column Storage

Settings for System Volumes, Flushing, Equilibration and Column Storage

This important screen allows you to specify the following settings (see Figure 43 on page 89 and Figure 45 on page 96):

- how solvent lines are flushed after a solvent change has occurred
- how a column is taken care of after it has been used
- how columns are equilibrated
- how the data files created during any of the above procedures are handled

**NOTE**

If the number of sequence lines required for analytical runs as specified by the campaign plus additional blank runs for flushing, column care and equilibration exceeds 1999, a warning is displayed, and you cannot proceed until either the range or dimensions of the screening campaign is reduced. This can be accomplished, for example, by deselecting a dimension such as temperature screening, or reducing the range of a dimension such as fewer gradients or temperatures.

Alternatively, but usually not recommended, you can also deselect flushing, column storage or column equilibration. This takes only one sample into account, if you plan to have more than one sample, you might get another warning during sample set up and need to further reduce the number of sequence entries.

**Defining System Volumes**

First, the volumes of the system that have to be flushed with solvents have to be defined (see Figure 43 on page 89). These settings have to be only entered once and will be stored for following screening campaigns as user defined settings, but they can be altered any time. If an external solvent selection valve is connected to a solvent channel of the pump the position of the degasser has to be selected. Two choices are available - before the solvent selection valve or after the solvent selection valve. In the later case it is necessary that the volume of the degasser chamber will be flushed. For the G1322A degasser, this volume can be significant.

**NOTE**

If two external solvent selection valves are configured in the system, only degassing either before or after the external solvent selection valves is supported. A configuration with one valve before the degasser and one after it is not supported.
Depending on the availability of an external solvent selection system and the selected position of the degasser, different system diagrams will be shown and different entries have to be made. Move with the mouse over the entry boxes and the corresponding volumes in the system diagram will be highlighted.

![Figure 43 Setting of the volumes in the system](image)

The following volumes are required:

- volume from degasser to mixing point of the pump (excluding the degasser chamber) - this volume will be flushed with 100 % of the corresponding solvent
- volume from solvent selection valve to degasser (including the degasser chamber) – this volume can be flushed with 100 % of the corresponding solvent
- volume from the mixing point of the pump to the column inlet - this volume will be flushed with the composition of the following analytical method

Table 4 on page 90 and Table 5 on page 91 gives you some guidelines for the volumes of different parts of a system.
## Table 4  Typical internal volumes of parts that need to be flushed for a solvent change (for pumps)

<table>
<thead>
<tr>
<th>Pump</th>
<th>Mixer</th>
<th>Damper</th>
<th>Internal Volume</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1311A</td>
<td>yes</td>
<td>yes</td>
<td>850 µL</td>
<td>at 200 bar</td>
</tr>
<tr>
<td>G1311B</td>
<td>yes</td>
<td>yes</td>
<td>850 µL</td>
<td>at 300 bar</td>
</tr>
<tr>
<td>G1312A</td>
<td>yes</td>
<td>yes</td>
<td>850 µL</td>
<td>at 200 bar</td>
</tr>
<tr>
<td>G1312B</td>
<td>yes</td>
<td>no</td>
<td>860 µL</td>
<td>at 300 bar</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>yes</td>
<td>220 µL</td>
<td>at 300 bar</td>
</tr>
<tr>
<td>G4220A/B</td>
<td>no</td>
<td>no</td>
<td>33 µL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>35 µL</td>
<td>no</td>
<td>75 µL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 µL</td>
<td>no</td>
<td>160 µL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>380 µL</td>
<td>no</td>
<td>390 µL</td>
<td></td>
</tr>
<tr>
<td>G7120A</td>
<td>no</td>
<td>no</td>
<td>33 µL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>35 µL</td>
<td>no</td>
<td>75 µL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 µL</td>
<td>no</td>
<td>160 µL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>380 µL</td>
<td>no</td>
<td>390 µL</td>
<td></td>
</tr>
<tr>
<td>G4204A</td>
<td>no</td>
<td>no</td>
<td>450 µL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>380 µL</td>
<td>no</td>
<td>840 µL</td>
<td></td>
</tr>
<tr>
<td>G7104A</td>
<td>no</td>
<td>no</td>
<td>470 µL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>380 µL</td>
<td>no</td>
<td>865 µL</td>
<td></td>
</tr>
</tbody>
</table>
Table 5  Typical internal volumes of parts that need to be flushed for a solvent change

<table>
<thead>
<tr>
<th>Part</th>
<th>Internal Volume (geometric)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Built-in degassing unit</td>
<td>1.5 mL</td>
<td></td>
</tr>
<tr>
<td>(G4220A/B, G4204A, G1311B pumps)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G4225A Degasser</td>
<td>0.45 mL</td>
<td>per channel</td>
</tr>
<tr>
<td>Solvent tubing 1.5 mm ID</td>
<td>1.8 mL/m</td>
<td></td>
</tr>
<tr>
<td>Capillary 0.17 mm ID</td>
<td>0.02 mL/m</td>
<td></td>
</tr>
<tr>
<td>Capillary 0.12 mm ID</td>
<td>0.01 mL/m</td>
<td></td>
</tr>
<tr>
<td><strong>Sampler (hydraulic volume without seat):</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1329AB, G1313A, G1367ABCDE, G5667A,</td>
<td>0.3 mL</td>
<td>Syringe volume 100 µL</td>
</tr>
<tr>
<td>G4226A, G1120 LC System Sampler,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1220 LC/VL System Sampler</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1367D, G1377A</td>
<td>0.142 mL</td>
<td>Syringe volume 40 µL</td>
</tr>
<tr>
<td>G1367E</td>
<td>0.118 mL</td>
<td>Syringe volume 40 µL</td>
</tr>
<tr>
<td>G4226A</td>
<td>0.08 mL</td>
<td>Syringe volume 20 µL</td>
</tr>
<tr>
<td>G4226A</td>
<td>0.118 mL</td>
<td>Syringe volume 40 µL</td>
</tr>
<tr>
<td>G7167AB, G7129AB</td>
<td>0.04 mL</td>
<td>Syringe volume 40 µL</td>
</tr>
<tr>
<td>G7167AB, G7129AB</td>
<td>0.0615 mL</td>
<td>Syringe volume 100 µL</td>
</tr>
<tr>
<td>G7167AB, G7129A</td>
<td>0.1772 mL</td>
<td>Syringe volume 900 µL</td>
</tr>
</tbody>
</table>

**NOTE**
Sampler: The seat volume has to be added as configured by the customer.
Example:

Degasser *after solvent selection valve*, system with a binary pump G7120A / 100 μL mixer and the G7167B autosampler with 100 μL Syringe:

Volume from SSV to degasser, incl. degasser:

Ca. 30 cm tubing  
Built-in degaasing unit  

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca. 30 cm tubing</td>
<td>0.54</td>
</tr>
<tr>
<td>Built-in degaasing unit</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>2.04</td>
</tr>
</tbody>
</table>

Volume from degasser to mixer:

20 cm tubing  
Pump head

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 cm tubing</td>
<td>0.36</td>
</tr>
<tr>
<td>Pump head</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>0.42</td>
</tr>
</tbody>
</table>

Volume from mixer to column, incl. mixer:

Mixer  
Capillaries ca. 1.2 m 0.12 ID  
Autosampler

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixer</td>
<td>0.10</td>
</tr>
<tr>
<td>Capillaries ca. 1.2 m 0.12 ID</td>
<td>0.013</td>
</tr>
<tr>
<td>Autosampler</td>
<td>0.0615</td>
</tr>
<tr>
<td></td>
<td>0.1745</td>
</tr>
</tbody>
</table>

In case more than one solvent line needs to be flushed, for example at initial solvent line flush at the very beginning of a campaign, the pump will be set to a composition that reflects the volume ratios of the different channels (e.g. high percentage for a solvent that is delivered through an external solvent selection valve and a degasser after the SSV and low percentage for the remaining solvents that are directly attached to the degasser).

The void volume is usually taken from the column data base. In few special cases it can be necessary to enter the void volume of the column as well.
Flushing Conditions

If you have included solvent screening, or you want to store your columns with a storage solvent at the end of their usage, solvents have to be exchanged in the system. It is highly recommended not to deselect the flushing option, as this can lead to non-reproducible results. However, in a few cases, it might be appropriate to deselect this option; for example, when the volumes to be flushed are very low, high flow rates and equilibration conditions in the method would cover the times needed to flush the solvent delivery system.

First, it is necessary to select the flushing solvent (see Figure 44 on page 94). Usually, this is the solvent of the next method, but in some cases, it might be necessary to flush the system first with a neutral solvent followed by the solvent of the next method. Cases that require an intermediate neutral solvent might be immiscible solvents or any solvent combinations that might cause precipitation of buffers. If you fear such problems, you can select one of the available solvent from your system, and the complete system (including the column) is flushed first with this solvent and then with the solvent of the following analytical run or a solvent that was selected for a column care procedure (see “Column Storage Procedures” on page 98).

NOTE

Note that additional flushing with a neutral solvent doubles the required flushing time.
The settings for the flushing conditions depend on the availability of a waste and/or bypass line in the system. With a waste line, a much higher flow can be applied giving reduced flush time.

If only a bypass line is available, a lower flow rate as with a waste line might be considered, depending on the detector in use. For example, the backpressure generated at high flow rates by the flow cell might be too high for an FLD-detector, and some detectors such as mass spectrometers or ELSD detectors typically have maximum allowed flow rates in the range of 1 – 2 mL/min.

If no waste or bypass line is available the flow needs to go through the column. You must set an appropriate flow rate, taking into account different viscosities and possible immiscibility of the new solvent with the solvent residing in the column.

Finally, the number of flush volumes (n) needs to be entered. The calculated flush time is (n) times the flush volume divided by the flow rate. A value of at least 5 is advisable to achieve a thorough flushing.

If flushing is selected, the solvent lines used in the first analysis are flushed at the beginning of a sequence generated by the Agilent Method Scouting Wizard to ensure proper starting conditions.
Column Equilibration Procedure

The column equilibration procedure is applied after the change of a column, the change of a solvent, after the change of the temperature or the change of a gradient. It is not applied between multiple uses of the same method for multiple injections or multiple samples or both.

If equilibration is selected, a column is treated with the conditions of the following analytical run for the specified time. The equilibration time can be set to a fixed value or can be calculated depending on the column void volumes. The column volume used for some of the calculations is taken from the column data base in ChemStation, and correct settings are assumed. To calculate the void volume of a column use the following equation:

\[ V_m = \pi \left( \frac{d_c}{2} \right)^2 \cdot L_c \cdot \frac{\varepsilon_t}{1000} \]

where

- \( V_m \) column volume [mL]
- \( d_c \) internal diameter [mm]
- \( L_c \) column length in [mm]
- \( \varepsilon_t \) total porosity

Typically, the total porosity, which is the fraction of the column that is not taken up by the stationary phase and accessible to the mobile phase, is in the range of 0.6 – 0.8; for example, an Agilent Zorbax reversed phase column has a total porosity of ca. 0.6. In this case, a minimum of 5 column void volumes should be exchanged to ensure a proper equilibration, but much higher values might be appropriate, depending on the type of columns used.

The value set here is used for all columns. If a certain column needs significantly longer equilibration times, you could deliberately set the value in the column data base to a higher value than the physical value to achieve a longer flushing time for this column.

The use of the column equilibration procedure is highly recommended.
Figure 45  Settings for equilibration conditions

If the equilibration time has been specified directly in minutes, and not calculated from the column void volume, and time scaling has been defined on the Set up column screening page, the respective column length ratio, r, is applied to scale the time as given in the user interface.

The equilibration time specified here refers to columns without extended equilibration factor.

The actual equilibration time is a multiple of the time specified here and the column equilibration factor. The actual equilibration time is displayed in the Sequence tab on the Summary page.

The equilibration factor $f_e$ applies to the equilibration time $t_e$ only. If a flush tubing volume flow time $t_{flush}$ has been added to the equilibration time $t_e$, the extended equilibration time $t_{ext}$ computes to:

$$t_{ext} = f_e \cdot t_e + t_{flush}$$

When the column length ratio, $r$, is also applied, the equation becomes:

$$t_{ext} = r \cdot f_e \cdot t_e + t_{flush}$$
If column equilibration is not selected, it is recommended that the gradients used in the screening campaign contain an adequate equilibration time, and that the sequence starts with a blank sample. Otherwise, the first run will not have reproducible results. Also, if multiple injections are planned with the same method, either the method must contain a post-time or the gradient must include a programmed time for equilibration.

Single use of an analytical method

Multiple use of an analytical method

Figure 46  Top: an analytical method is used only once, then new conditions are applied. The Equilibration procedure ensures proper column equilibration under the new conditions. Bottom: multiple injections or multiple samples are planned. Since the Equilibration procedure is applied only after a change of column, solvents, temperatures or gradient, the method must contain a post-time (or the gradient must include a programmed equilibration period) to ensure proper equilibration in-between the multiple analysis.
Column Storage Procedures

After a column has been used in an analysis, it might be advisable to flush the column with another solvent than the one used for the analysis. A typical example might be after analysis with high buffer concentrations, see Figure 47 on page 98. Different choices of flush solvents are available:

- the starting conditions of the current method, which might be of use if the gradient ends with a high concentration of organic solvent, for example,
- an additional care solvent provided on a separate channel of the solvent delivery system, which is probably the regular case.

**NOTE**
The flushing option should not be deselected.

You can make different settings for the flow rates and the flush times to ensure that a proper solvent exchange inside the column has occurred.

![Figure 47 Settings for column storage conditions](image)

If an extra column storage solvent is used and the flow has been defined directly, and not by the injection/base method, and flow scaling has been
defined on the **Set up column screening** page, the respective column squared-diameter ratio is applied to scale the flow as given in the user interface.

If the time has been specified directly in minutes, and not by column void volume, and time scaling has been defined on the **Set up column screening** page, the respective column length ratio, \( r \), is applied to scale the time as given in the user interface.

The column storage time as specified here refers to columns without extended storage factor.

The actual column storage time is a multiple of the time as specified on this screen and the column storage factor as specified on the **Set up column screening** page. The actual column storage time is displayed in the **Sequence** tab on the **Summary** page.

The equilibration factor \( f_e \) applies to the column storage time \( t_{cs} \) only. If a flush tubing volume flow time \( t_{\text{flush}} \) has been added to the column storage time \( t_{cs} \), then the extended column storage time \( t_{ext} \) computes to:

\[
t_{ext} = f_e \times t_{cs} + t_{\text{flush}}
\]

When the column length ratio, \( r \), is also applied, the equation becomes:

\[
t_{ext} = r \times f_e \times t_{cs} + t_{\text{flush}}
\]

**Blank-Run Data File Handling**

When this check box is marked, data files generated during flushing, column care or column equilibration runs are deleted automatically. The deletion of these data files is recommended in order to keep the amount of data generated low, but the files might be helpful for problem solving (for example, if compounds are eluted after the end of the analytical run because of improper gradient conditions).
Setting Up the Samples

The samples to be analyzed under the different method conditions are specified in this screen (see Figure 48 on page 101). At the top, you specify the total volume [μL] of a single vial (or well of a microtiter plate).

**NOTE**

Only one sort of sample vial must be used.

In the central table, you enter a sample name, select the vial-positions in the graphical representation of the autosampler plates, and specify the individual injection volume and the number of injections per sample and number of repetitions. The total injection volume per sample and condition is calculated (repetitions x injection volume), the total sample volume for the campaign is also calculated (scouting conditions x repetitions x injection volume), and finally the number of required vials is calculated (scouting conditions x repetitions x injection volume / volume of a single vial).

**NOTE**

For the number of required vials a safety margin of 10 % of the required volume is taken into account.

If either the specified vial positions do not match the amount of vials required, or the vial positions of different samples overlap, a warning is displayed, and you cannot proceed until the faulty condition is cleared. You might also get a warning if the total number of sequence lines including all flushing, column storage and column equilibration methods exceeds 1999 lines.

More sample lines can be added by clicking on the Add button.
Figure 48  Set up the samples
Summary

The last screen of the wizard gives you detailed information on the specified method scouting campaign (see Figure 49 on page 103).

The **Description** tab gives general information:

- number of columns, solvents, gradients and temperatures
- name and path of the selected base method
- column storage and equilibration as well as flushing procedure
- sample names and required total sample volumes
- name and path of the sequence created

The **Sequence** tab shows details of the sequence that will be created:

- the complete sequence is shown including all flush, equilibration and column care lines (these are color coded)
- net run time (calculated without system overhead times)
- estimated run time (calculated with a general overhead factor plus a temperature-dependent factor that takes heating and cooling periods into account)
- number of equilibration, column storage and flush sequence lines
- number of column and solvent changes

The **Solvent Usage** tab gives an estimate of the required solvent volumes based on the type of solvent, the estimated run time for the specific solvent and the flow rate of the different methods using the specific solvent.

All estimated solvent volume is summed to give a total waste volume, which is shown in the last row of the table. If your pump supports a waste bottle and your system is online, the computed waste volume is compared with the waste bottle capacity and is color-coded accordingly.

When you click the **Finish** button, the sequence is automatically set up according to the inputs given in the previous screens.
When you have completed the campaign set up, you can load and start the sequence. Open the **Sequence** menu, select **Load Sequence** and browse to the specified path for the campaigns. Highlight the appropriate campaign and press **OK**. The campaign is now loaded as a standard ChemStation sequence. You can specify the data directory (for example, as sub-directory in the campaign directory) by opening the **Sequence** menu and selecting **Sequence Parameter**; data-file naming conventions and sequence shutdown parameters can also be specified.

**NOTE**
To avoid negative effects on the results (for example caused by improper flushing or equilibration), take the utmost care when making changes to the sequence (for example, deleting sequence lines). It is advisable to make such changes in the Method Scouting Wizard.
The Method Scouting Wizard automatically enters a detailed sample information field giving information about the column, the solvent combination, the gradient and the temperature used for the specific data file. All other standard report fields for sample information, method details etc. are available as usual.

For even more advanced and automated method optimization based on chromatographic data and considering aspects of quality by design (QbD), Agilent has partnered with:

- ACD Labs
- ChromSword
- S-Matrix
6 Method Development Strategy

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This chapter provides information on method development strategy, concerning LC and LC/MS columns selection, pH and mobile phase.
Different classes of compounds require different separation mechanisms. The column selection guide in Figure 50 on page 107 allows you to find a suitable separation mechanism for a very broad range of chemical and biochemical compounds. To use the column selection guide, follow the path for your analyte and mobile phase.
Figure 50  Find the right separation mechanism for a new sample
HPLC columns consist of two parts, the column chemistry and the hardware. Both parts have a significant influence on the outcome of the final method, see Figure 51 on page 108. Table 6 on page 108 gives you an idea for the optimal column internal diameter for your application. Note that the Agilent 1290 Infinity II Method Development Solution offers optimized capillary kits for all application ranges.

**Figure 51** How column characteristics influence the separation

**Table 6** Column choice relative to application objective

<table>
<thead>
<tr>
<th>Application Objective</th>
<th>Column Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Save solvent; special low-volume instrumentation is available</td>
<td>2.1</td>
</tr>
<tr>
<td>Special detectors, e.g., mass spec</td>
<td>2.1</td>
</tr>
<tr>
<td>High sensitivity, limited sample</td>
<td>2.1</td>
</tr>
<tr>
<td>Save solvent; standard HPLC equipment available, LC/MS</td>
<td>3.0</td>
</tr>
<tr>
<td>Standard separations</td>
<td>4.6</td>
</tr>
</tbody>
</table>
Pore Size Selection

If the solute molecular weight is less than about 5000 Da, choose a column packing with small pore size (60 – 120 Å). Otherwise, use column packing with 300 Å pore size.

Particle Size Selection

The standard particle size for HPLC columns is still 5 μm, with 3.5 μm now dominant for method development. If high-speed analyses or higher resolution analyses are required, packings with 1.8 μm and 3.5 μm particles are recommended. Shorter columns with these particles can produce faster high-resolution separations, and the 1.8 μm particle size in Rapid Resolution HT columns provide the highest efficiency. The 3.5 μm particle size operates at a routine operating pressure and can be used on all LCs. Columns with 1.8 μm particle size can be used on optimized standard LCs if they are short (50 mm and below) and/or have internal diameters of at least 3.0 mm. Longer and narrower columns usually require a higher pressure LC (one supporting pressures greater than 400 bar).

Superficially Porous Particles

Poroshell columns are so-called superficially porous particle (SPP) columns. In contrast to totally porous silica columns, these SPP columns have a solid core (1.7 μm in diameter) and a porous silica layer (0.5 μm thickness) surrounding it. Speed and resolution of Poroshell columns are comparable to sub-two micron columns with up to 50 % less backpressure. Poroshell columns have enjoyed a recent resurgence in smaller particle sizes than the older 'pellicular' particle columns. The current interest in this technology is driven by its re-introduction in smaller particle sizes, such as the sub 3 micron sizes, for use in typical small molecule reversed phase separations.
Silica Type and Bonded Phase

Silica Type

Agilent ZORBAX reversed phase columns use three different types of porous silica microspheres:

- The original ZORBAX SIL,
- ZORBAX Rx-SIL and
- modified ZORBAX Rx-SIL.

ZORBAX Rx-SIL and modified ZORBAX Rx-SIL are highly purified and less acidic than the original ZORBAX SIL. Less acidic silica means less potential for interaction between the analyte and silanol groups on the silica surface, especially if the solutes are basic, and contributes to improved peak shape.

**NOTE**
For new method development, we strongly recommend using reversed-phase products based on modified ZORBAX Rx-SIL (Eclipse Plus) and ZORBAX Rx-SIL (Eclipse, StableBond etc.).

Bonded Phase

A good first choice for bonded phase is C18 or C8. If the sample solutes of interest are not adequately separated on these columns, CN and Phenyl columns may offer significant differences in selectivity from the straight-chain alkyl phases to effect the separation.

In general, larger solutes, such as proteins, are best separated on short-chain reversed-phase columns (C3, CN) and peptides and small molecules are separated on longer-chain columns (C8, C18). There are many cases, however, where this conventional wisdom does not apply. For example, peptides can also be effectively separated using short-chain columns, and hydrophobic peptides can show better recovery on longer-chain phases.

Therefore, it is best to initially select a phase in the middle of the hydrophobic spectrum (e.g., C8), then change to a more hydrophobic phase or more hydrophilic phase depending on initial results and solubility properties of your sample.
### Table 7  Quick guide to ZORBAX reversed-phase bonded phases

<table>
<thead>
<tr>
<th>Type</th>
<th>Recommended uses and applications</th>
</tr>
</thead>
</table>
| Eclipse Plus\(^1\)    | • Excellent first choice for method development  
                      • Different selectivity choices for flexible method development  
                      • Long life from pH 2-9 for reliable separations of basic, acidic and neutral compounds  
                      • Superior peak shape with basic compounds  
                      • Rigorous QA/QC testing for greater long-term reproducibility                                                                                  |
| Eclipse XDB           | • Four selectivity choices for flexible method development  
                      • High performance over a wide pH range, pH 2 – 9  
                      • Good peak shape for acids, bases and neutrals  
                      • Long lifetime with extra dense bonding and double endcapping                                                                               |
| StableBond (SB)\(^1\) | • Basic, acidic, neutral compounds  
                      • Exceptional stability at low pH  
                      • Use of high temperature (up to 90 °C for C18, 80 °C for C8, C3, Phenyl, CN, and Aq) and low pH as an added selectivity tool  
                      • Widest selection of bonded phases for different selectivity (C18, C8, C3, CN, Phenyl, Aq)  
                      • Uses mobile phases for LC/MS with formic acid, acetic acid, or TFA  
                      • Uses mobile phases with TFA for peptide and protein separation                                                                               |
| ZORBAX Rx             | • General separation of basic, acidic and neutral compounds at low pH with different selectivity than SB columns  
                      • Rx-C8 is the same as SB-C8                                                                                                                      |
| Bonus-RP\(^1\)        | • Separating basic compounds in higher aqueous mobile phases  
                      • General separation of basic, neutral, acidic compounds at mid-range pH or low pH; especially stable at low pH  
                      • Separating peptides for different selectivity                                                                                                 |
| Extend-C18\(^1\)      | • Separating basic compounds above their pKa in free base form; separation of basic, acidic, neutral compounds at high pH; up to pH 11.5  
                      • Uses ammonium hydroxide as mobile phase additive with LC/MS with small molecules or peptides  
                      • Separating at high, mid-range and low pH for selectivity changes                                                                             |

\(^1\) Stationary phases are used in the method development column kits optionally available with an Agilent 1290 Infinity II Method Development Solution
pH and Mobile Phase

The choice of mobile phase for a reversed-phase system starts with the selection of the organic modifier.

- Selectivity differences and sample retention vary significantly among mobile phases containing acetonitrile, methanol, and tetrahydrofuran (THF).
- Sample solubility is likely to differ in such solvents and dictates the use of a specific solvent or solvents.
- UV detection at certain wavelengths is not possible with certain modifiers (e.g., methanol at 200 nm).
- Both pH and ionic strength of the aqueous portion of mobile phases are important parameters in developing rugged methods that are not sensitive to small variations in conditions.

With ionic compounds, retention of typical species shows significant changes with pH. It is very important to control pH in such reversed-phase systems to stabilize retention and band spacing.

A pH set between 2 and 4 generally provides the most stable conditions for retention vs. small changes in pH and this pH is recommended for starting method development for most samples, including basic compounds and typical weak acids.

**Method development from pH 1-12**

Chromatographic resolution between two or more peaks depends upon three factors: column efficiency, selectivity, and retention. With ionizable analytes – bases and acids – all of these factors change dramatically with pH. For example, retention can be improved by changing the separation pH, so that analytes are separated in their non-ionized form.

Changes in mobile phase pH also improve column efficiency because the ionization of the analyte and the residual silanols can both be altered. This minimizes secondary interactions between analytes and the silica surface that cause poor peak shape. Achieving optimum resolution can also require changing the mobile phase pH.
The following method development strategy explains how this is done with superior column lifetime.

Low, mid, and high pH are the three general regions for chromatographic separations as shown in Figure 52 on page 113. This figure highlights the benefits of performing separations of ionizable analytes in each pH region. Method development proceeds by investigating chromatographic separations first at low pH and then at higher pH until optimum results are achieved. The ideal column is available for each pH region.

**Figure 52** Three pH regions for HPLC separations of basic compounds. This figure represents the retention behavior of one basic analyte with respect to pKa and pH. Analyte pKa is 6.5.
Method Development Strategy

pH and Mobile Phase

Table 8  Characteristics of the three pH-regions

<table>
<thead>
<tr>
<th>Low pH &lt; 3 – Region A</th>
<th>Mid pH 7 – Region B</th>
<th>High pH &gt; 9 – Region C</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Start method development at low pH, where silanols on a RPHPLC column are protonated. This minimizes peak tailing by eliminating silanol/base interactions.</td>
<td>• Develop methods at pHs at least 1 pH unit above or below the pKa to minimize changes in retention with small changes in pH.</td>
<td>• In this region, basic compounds may be in their free base form.</td>
</tr>
<tr>
<td>• At low pH, basic compounds are positively charged and their retention may be reduced.</td>
<td>• Some silica surface SiOH groups become SiO⁻ above pH 4 to 5; tailing interactions may be possible.</td>
<td>• Increased retention and resolution of basic compounds are likely.</td>
</tr>
<tr>
<td>• Acidic compounds may be protonated and have increased retention.</td>
<td>• Minimize interactions by selecting a well-designed endcapped column, using additives such as TEA (triethylamine) (less desirable) or using &quot;polar-linked&quot; bonded phases.</td>
<td>• Retention changes little in this region, thus robust methods can be developed.</td>
</tr>
<tr>
<td>• Retention times are usually stable with small changes in pH, producing a robust method.</td>
<td>• Silica breakdown is prevented by innovative bonding chemistry, heavy endcapping, and use of Rx-SIL.</td>
<td>• Some silica surface SiOH groups become SiO⁻ above pH 4 to 5; tailing interactions may be possible.</td>
</tr>
<tr>
<td>• Volatile mobile phase additives, such as formic acid or trifluoroacetic acid (TFA), are often used at low pH with LC/MS.</td>
<td>• In this region, basic compounds may be in their free base form.</td>
<td>• Minimize interactions by selecting a well-designed endcapped column, using additives such as TEA (triethylamine) (less desirable) or using &quot;polar-linked&quot; bonded phases.</td>
</tr>
<tr>
<td>• In this region, basic compounds may be in their free base form.</td>
<td>• Increased retention and resolution of basic compounds are likely.</td>
<td>• Silica breakdown is prevented by innovative bonding chemistry, heavy endcapping, and use of Rx-SIL.</td>
</tr>
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<td>• Increased retention and resolution of basic compounds are likely.</td>
<td>• Silica breakdown is prevented by innovative bonding chemistry, heavy endcapping, and use of Rx-SIL.</td>
<td>• Retention changes little in this region, thus robust methods can be developed.</td>
</tr>
<tr>
<td>• Silica breakdown is prevented by innovative bonding chemistry, heavy endcapping, and use of Rx-SIL.</td>
<td>• Ammonium hydroxide is an excellent volatile mobile phase modifier at high pH.</td>
<td>• Some silica surface SiOH groups become SiO⁻ above pH 4 to 5; tailing interactions may be possible.</td>
</tr>
</tbody>
</table>

Start Method Development at Low pH (pH 2-3)

With so many column choices available, how do you know where to start your method development?

The recommended starting point for method development is to use a buffered low pH mobile phase – around pH 2 – 3. Using a low pH mobile phase most often results in the best peak shape for basic compounds on silica-based columns. At low pH, the silanols on the silica are fully protonated so positively charged basic compounds do not interact strongly. The result is good peak shape. Many acidic compounds are noncharged, maximizing their retention at low pH. These observations are key advantages to method development at low pH.
For standard analytical work, start method development with acetonitrile as the mobile phase organic modifier and 20 – 50 mM phosphate buffer (pH 2 – 3) as the aqueous component for non-LC/MS applications. These conditions provide good pH control, necessary for the most reproducible analyses of ionizable compounds. For LC/MS applications formic acid or TFA are good mobile phase additives for low pH.

**Choose Agilent ZORBAX Eclipse Plus first for best peak shape**

Select ZORBAX Eclipse Plus C18 or C8 columns first for method development at low pH. Eclipse Plus columns are the newest addition to the Eclipse family and use improved silica and bonding technologies to provide good peak shape for basic compounds. Eclipse Plus columns can be used from pH 2 – 9, providing flexibility for method development. They are stable down to pH 2 making them an ideal choice for initial method development.

**Optimize solvents and bonded phases at low pH**

The initial method development steps may lead very quickly to a satisfactory separation. But if more optimization is needed, acetonitrile can be replaced by methanol or tetrahydrofuran and the separation reoptimized. This step may lead to a satisfactory solution, but if further optimization of selectivity is needed, the column bonded phase can be changed.

At low pH there are many bonded phase choices available for optimization. These include the Eclipse Plus phases as well as the Eclipse XDB family with C18, C8, Phenyl and CN. Alternate choices include six different StableBond bonded phases: SB-C18, SB-C8, SB-Phenyl, SB-CN, SB-C3, and SB-Aq. It may be necessary at low pH to improve the retention of acidic compounds. For these situations, lower the pH even further, down to pH 1 – 2, and use StableBond columns. These columns provide the greatest stability at very low pH and provide many selectivity options for achieving the highest resolution separations.
Method Development at Mid pH (4-9) - Agilent ZORBAX Eclipse Plus

There are some samples that may not be resolved at low pH or may have better solubility and stability at mid pH. The Eclipse Plus C18 column can be used for method development in the mid pH range. The Eclipse Plus column is stable up to pH 9 so it is equally reliable at mid pH. These double endcapped columns have two key advantages: good peak shape at low and mid pH, and sufficient bonded phase density to protect the column from silica degradation from pH 6 – 9.

At mid pH, basic compounds (e.g., amines) may still have a positive charge, and the silanols on the silica surface may have a negative charge. Therefore, the best peak shape at mid pH is achieved when as many silanols as possible are covered. This makes the Eclipse Plus C18 the best starting choice for a column at mid pH. Phosphate buffer is usually the first choice for mobile phase modifier at pH 7 because its buffer range is pH 6.1 – 8.1. A second choice for mid pH is acetate buffer, since it buffers from pH 3.8 – 5.8 and its volatility makes it a good choice for LC/MS compatibility.

Alternate selectivities – Agilent ZORBAX Eclipse Plus Phenyl-Hexyl, Eclipse XDB-Phenyl, CN and Bonus-RP

The method development process at mid pH mimics the process at low pH with optimization of the organic modifier and selecting an alternate bonded phase if resolution is not achieved after that step. The alternate bonded phases at mid pH are the Eclipse Plus Phenyl-Hexyl, Eclipse XDB-Phenyl, Eclipse XDB-CN and Bonus-RP. They provide very different selectivities for many samples and the method development process is followed again. The Bonus-RP column has a polar embedded amide group that provides different selectivity for many samples, provides good peak shape for basic compounds and allows the column to be used with up to 100 % aqueous mobile phases.
Method Development at High pH (pH 9-12) - Choose Agilent ZORBAX Extend-C18 Columns

At low or mid pH, some separations of basic compounds may still not have enough retention or the desired selectivity. For these samples, high pH separations may be appropriate. Until recently, high pH separations on silica-based columns were avoided because of short column lifetimes, due to dissolution of the underlying silica gel. Newer column technologies, such as the ZORBAX Extend-C18, can protect the silica from dissolution, so that a reasonable column lifetime can be achieved and the selectivity advantages of high pH can be explored.

The mobile phase buffer choices at high pH with the Extend-C18 column are organic buffers such as triethylamine and ammonium hydroxide. These buffers are best used with methanol as the organic modifier to extend the column lifetime at high pH.
Available Column Kits

As a starting point for method development six different column kits, each with three columns, are available as options to the G4230A or B Valve Kits (for the low pressure valve kit G4230A only four column kits are available). You have the choice between RRHT and RR columns, different internal diameters and different application focuses.

Method Development Column Kits Based on Changing Selectivity

Columns included are:

- Eclipse Plus C18,
- Eclipse Plus Phenyl-Hexyl and
- Bonus-RP (polar alkyl amide)

These three columns provide dramatic differences in selectivity, and can alter the retention order and overall retention of acidic and basic compounds as well as aromatic and non-aromatic compounds, that is, the typical sample.

RRHT Selectivity Method Development Kit (p/n 5190-1431)

RRHT Selectivity Method Development Kit, 2.1 mm i.d. (5190-1431) contains:

<table>
<thead>
<tr>
<th>p/n</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>959741-902</td>
<td>Eclipse Plus C18, 2.1 x 50 mm, 1.8 µm, 600 bar</td>
</tr>
<tr>
<td>959741-912</td>
<td>Eclipse Plus Ph-Hex, 2.1 x 50 mm, 1.8 µm, 600 bar</td>
</tr>
<tr>
<td>827768-901</td>
<td>Bonus-RP, 2.1 x 50 mm, 1.8 µm, 600 bar</td>
</tr>
</tbody>
</table>
**RRHT Selectivity Method Development Kit (p/n 5190-1433)**

RRHT Selectivity Method Development Kit, 4.6 mm i.d. (5190-1433) contains:

<table>
<thead>
<tr>
<th>p/n</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>959941-902</td>
<td>Eclipse Plus C18, 4.6 x 50 mm, 1.8 µm, 600 bar</td>
</tr>
<tr>
<td>959941-912</td>
<td>Eclipse Plus Ph-Hex, 4.6 x 50 mm, 1.8 µm, 600 bar</td>
</tr>
<tr>
<td>827668-901</td>
<td>Bonus-RP, 4.6 x 50 mm, 1.8 µm, 600 bar</td>
</tr>
</tbody>
</table>

**Rapid Resolution Selectivity Method Development Kit (5190-1435)**

Rapid Resolution Selectivity Method Development Kit (5190-1435) contains:

<table>
<thead>
<tr>
<th>p/n</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>959961-902</td>
<td>Eclipse Plus C18, 4.6 x 100 mm, 3.5 µm</td>
</tr>
<tr>
<td>959961-912</td>
<td>Eclipse Plus Phenyl-Hexyl, 4.6 x 100 mm, 3.5 µm</td>
</tr>
<tr>
<td>864668-901</td>
<td>Zorbax Bonus-RP, 4.6 x 100 mm, 3.5 µm</td>
</tr>
</tbody>
</table>

**Method Development Column Kits Based on pH Variation**

Columns included are:

- Eclipse Plus C18,
- SB-C18 and
- Extend-C18

These three C18 columns provide differences in selectivity and follow the Agilent recommended method development process for screening different pH-values. The recommended starting point is pH 2 – 3 with an Eclipse Plus C18 column. This covers pH 2 – 9. The StableBond-C18 is ideal for going below pH 2 and using a non-endcapped column for alternate selectivity whereas the Extend-C18 is ideal for evaluating method selectivity above pH 9.
RRHT pH Method Development Kit (p/n 5190-1432)
RRHT pH Method Development Kit, 2.1 mm i.d. (5190-1432) contains:

<table>
<thead>
<tr>
<th>p/n</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>959741-902</td>
<td>Eclipse Plus C18, 2.1 x 50 mm, 1.8 µm, 600 bar</td>
</tr>
<tr>
<td>827700-902</td>
<td>SB-C18, 2.1 x 50 mm, 1.8 µm, 600 bar</td>
</tr>
<tr>
<td>727700-902</td>
<td>Extend-C18, 2.1 x 50 mm, 1.8 µm, 600 bar</td>
</tr>
</tbody>
</table>

RRHT pH Method Development Kit (p/n 5190-1434)
RRHT pH Method Development Kit, 4.6 mm i.d. (5190-1434) contains:

<table>
<thead>
<tr>
<th>p/n</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>959941-902</td>
<td>Eclipse Plus C18, 4.6 x 50 mm, 1.8 µm, 600 bar</td>
</tr>
<tr>
<td>827975-902</td>
<td>SB-C18, 4.6 x 50 mm, 1.8 µm, 600 bar</td>
</tr>
<tr>
<td>727975-902</td>
<td>Extend-C18, 4.6 x 50 mm, 1.8 µm, 600 bar</td>
</tr>
</tbody>
</table>

Rapid Resolution pH Method Development Kit (p/n 5190-1436)
Rapid Resolution pH Method Development Kit (5190-1436) contains:

<table>
<thead>
<tr>
<th>p/n</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>959961-902</td>
<td>Eclipse Plus C18, 4.6 x 100 mm, 3.5 µm</td>
</tr>
<tr>
<td>861953-902</td>
<td>SB-C18 Rapid Res, 4.6 x 100 mm, 3.5 µm</td>
</tr>
<tr>
<td>764953-902</td>
<td>Zorbax Extend C18, 4.6 x 100 mm, 3.5 µm</td>
</tr>
</tbody>
</table>
Recommended Method Development Workflow

Figure 53 on page 122 illustrates an easy to follow workflow for method development from low to high pH. For more information, refer to “LC and LC/MS Columns Selection” on page 106 and “pH and Mobile Phase” on page 112.
Figure 53  Recommended method development workflow
6 Method Development Strategy
Recommended Method Development Workflow
7
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This chapter provides addition information on safety, legal and web.
General Safety Information

The following general safety precautions must be observed during all phases of operation, service, and repair of this instrument. Failure to comply with these precautions or with specific warnings elsewhere in this manual violates safety standards of design, manufacture, and intended use of the instrument. Agilent Technologies assumes no liability for the customer’s failure to comply with these requirements.

**WARNING**

Ensure the proper usage of the equipment.
The protection provided by the equipment may be impaired.

⇒ The operator of this instrument is advised to use the equipment in a manner as specified in this manual.

Safety Standards

This is a Safety Class I instrument (provided with terminal for protective earthing) and has been manufactured and tested according to international safety standards.

General

Do not use this product in any manner not specified by the manufacturer. The protective features of this product may be impaired if it is used in a manner not specified in the operation instructions.
Before Applying Power

**WARNING**  
Wrong voltage range, frequency or cabling  
Personal injury or damage to the instrument  

➔ Verify that the voltage range and frequency of your power distribution matches to the power specification of the individual instrument.

➔ Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.

➔ Make all connections to the unit before applying power.

---

**NOTE**  
Note the instrument’s external markings described under “Safety Symbols” on page 130.

---

Ground the Instrument

**WARNING**  
Missing electrical ground  
Electrical shock  

➔ If your product is provided with a grounding type power plug, the instrument chassis and cover must be connected to an electrical ground to minimize shock hazard.

➔ The ground pin must be firmly connected to an electrical ground (safety ground) terminal at the power outlet. Any interruption of the protective (grounding) conductor or disconnection of the protective earth terminal will cause a potential shock hazard that could result in personal injury.
Do Not Operate in an Explosive Atmosphere

**WARNING**

- Presence of flammable gases or fumes
- Explosion hazard

→ Do not operate the instrument in the presence of flammable gases or fumes.

Do Not Remove the Instrument Cover

**WARNING**

- Instrument covers removed
- Electrical shock

→ Do Not Remove the Instrument Cover

→ Only Agilent authorized personnel are allowed to remove instrument covers. Always disconnect the power cables and any external circuits before removing the instrument cover.

Do Not Modify the Instrument

Do not install substitute parts or perform any unauthorized modification to the product. Return the product to an Agilent Sales and Service Office for service and repair to ensure that safety features are maintained.

In Case of Damage

**WARNING**

- Damage to the module
- Personal injury (for example electrical shock, intoxication)

→ Instruments that appear damaged or defective should be made inoperative and secured against unintended operation until they can be repaired by qualified service personnel.
Solvents

**WARNING**

Toxic, flammable and hazardous solvents, samples and reagents

The handling of solvents, samples and reagents can hold health and safety risks.

➔ When working with these substances observe appropriate safety procedures (for example by wearing goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the vendor, and follow good laboratory practice.

➔ Do not use solvents with an auto-ignition temperature below 200 °C (392 °F). Do not use solvents with a boiling point below 56 °C (133 °F).

➔ Avoid high vapor concentrations. Always keep the temperature in the sample compartment at least 25 K below the boiling point of the solvent used.

➔ Do not operate the instrument in an explosive atmosphere.

➔ Reduce the volume of substances to the minimum required for the analysis.

➔ Never exceed the maximum permissible volume of solvents (8 L) in the solvent cabinet. Do not use bottles that exceed the maximum permissible volume as specified in the usage guideline for solvent cabinet.

➔ Ground the waste container.

➔ Regularly check the filling level of the waste container. The residual free volume in the waste container must be large enough to collect the waste liquid.

➔ To achieve maximal safety, regularly check the tubing for correct installation.

---

**NOTE**

For details, see the usage guideline for the solvent cabinet. A printed copy of the guideline has been shipped with the solvent cabinet, electronic copies are available in the Agilent Information Center or via the Internet.
## Appendix

### General Safety Information

### Safety Symbols

<table>
<thead>
<tr>
<th>Table 9</th>
<th>Symbols</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Symbol" /></td>
<td>The apparatus is marked with this symbol when the user should refer to the instruction manual in order to protect risk of harm to the operator and to protect the apparatus against damage.</td>
</tr>
<tr>
<td><img src="image2" alt="Symbol" /></td>
<td>Indicates dangerous voltages.</td>
</tr>
<tr>
<td><img src="image3" alt="Symbol" /></td>
<td>Indicates a protected ground terminal.</td>
</tr>
<tr>
<td><img src="image4" alt="Symbol" /></td>
<td>The apparatus is marked with this symbol when hot surfaces are available and the user should not touch it when heated up.</td>
</tr>
<tr>
<td><img src="image5" alt="Symbol" /></td>
<td>Cooling unit is designed as vapor-compression refrigeration system. Contains fluorinated greenhouse gas (refrigerant) according to the Kyoto protocol. For specifications of refrigerant, charge capacity, carbon dioxide equivalent (CDE), and global warming potential (GWP) see instrument label.</td>
</tr>
<tr>
<td><img src="image6" alt="Symbol" /></td>
<td>Confirms that a manufactured product complies with all applicable European Community directives. The European Declaration of Conformity is available at: <a href="http://regulations.corporate.agilent.com/DoC/search.htm">http://regulations.corporate.agilent.com/DoC/search.htm</a></td>
</tr>
<tr>
<td><img src="image7" alt="Symbol" /></td>
<td>Manufacturing date.</td>
</tr>
<tr>
<td><img src="image8" alt="Symbol" /></td>
<td>Power symbol indicates On/Off. The apparatus is not completely disconnected from the mains supply when the power switch is in the Off position</td>
</tr>
<tr>
<td><img src="image9" alt="Symbol" /></td>
<td>Pacemaker Magnets could affect the functioning of pacemakers and implanted heart defibrillators. A pacemaker could switch into test mode and cause illness. A heart defibrillator may stop working. If you wear these devices keep at least 55 mm distance to magnets. Warn others who wear these devices from getting too close to magnets.</td>
</tr>
</tbody>
</table>
Magnetic field
Magnets produce a far-reaching, strong magnetic field. They could damage TVs and laptops, computer hard drives, credit and ATM cards, data storage media, mechanical watches, hearing aids and speakers. Keep magnets at least 25 mm away from devices and objects that could be damaged by strong magnetic fields.

Indicates a pinching or crushing hazard

Indicates a piercing or cutting hazard.

### A WARNING

alerts you to situations that could cause physical injury or death.

➔ Do not proceed beyond a warning until you have fully understood and met the indicated conditions.

### A CAUTION

alerts you to situations that could cause loss of data, or damage of equipment.

➔ Do not proceed beyond a caution until you have fully understood and met the indicated conditions.
Agilent Technologies on Internet

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http://www.agilent.com
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In This Book

This manual contains information on the Agilent 1290 Infinity II Method Development Solution.

The manual describes the following:

• Introduction,
• System setup and installation,
• configuration of the system using ChemStation,
• information on the Method Scouting Wizard,
• method development strategies.